	L #	Hits	Search Text	DBs	Time Stamp
1	L1	7	glnap$8	USPAT; US-PGPUB	2003/10/09 15:58
2	L2	347110	acetyl adj phosphate or acetylphosphate or acetate	USPAT; US-PGPUB	2003/10/09 15:59
3	L3	83953	promoter$1	USPAT; US-PGPUB	2003/10/09 15:59
4	L4	1448552	induc$8 or regulat$8 or activat$8 or modulat$8	USPAT; US-PGPUB	2003/10/09 15:59
5	L5	66	2 near10 4 near10 3	USPAT; US-PGPUB	2003/10/09 15:59
6	L6	10013	pps or phosphoenol adj pyruvate adj synthase$1	USPAT; US-PGPUB	2003/10/09 16:02
7	L7	98	6 near4 (gene$1 or sequence$1)	USPAT; US-PGPUB	2003/10/09 16:03
8	L8	24	7 same (overexpress$ or amplif$10 or increas$8)	USPAT; US-PGPUB	2003/10/09 16:04
9	L9	21	7 and 2	USPAT; US-PGPUB	2003/10/09 16:08
10	L10	7012	lycopene$1 or isoprenoid$1 or carotene$1 or astaxanthin$1	USPAT; US-PGPUB	2003/10/09 16:10
11	L11	1	6 same 10	USPAT; US-PGPUB	2003/10/09 16:10
12	L12	4074	10 and 2	USPAT; US-PGPUB	2003/10/09 16:11
13	L13	13	12 and 6	USPAT; US-PGPUB	2003/10/09 16:11

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	7	glnap$8	USPAT; US-PGPUB	2003/10/09 15:55

US-PAT-NO: 6598188

DOCUMENT-IDENTIFIER: US 6598188 B1

TITLE: Error-corrected codeword configuration and method

DATE-ISSUED: July 22, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Locke; Michael	Santa Clara	CA	N/A	N/A
Gulati; Kapil	Sunnyvale	CA	N/A	N/A

US-CL-CURRENT: 714/704, 714/759 , 714/779

ABSTRACT:

Modem selection of Reed-Solomon codeword configuration to maximize error-corrected data rate given channel analysis. A lookup table of maximal codeword size given parity bytes and channel MSE allows precomputation.

1 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	7	glnap$8	USPAT; US-PGPUB	2003/10/09 15:58
2	L2	347110	acetyl adj phosphate or acetylphosphate or acetate	USPAT; US-PGPUB	2003/10/09 15:59
3	L3	83953	promoter$1	USPAT; US-PGPUB	2003/10/09 15:59
4	L4	1448552	induc$8 or regulat$8 or activat$8 or modulat$8	USPAT; US-PGPUB	2003/10/09 15:59
5	L5	66	2 near10 4 near10 3	USPAT; US-PGPUB	2003/10/09 15:59

PGPUB-DOCUMENT-NUMBER: 20030187294

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030187294 A1

TITLE: Process for producing catalysts comprising nanosize metal particles on a porous support, in particular for the gas-phase oxidation of ethylene and acetic acid to give vinyl acetate

PUBLICATION-DATE: October 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hagemeyer, Alfred	Frankfurt		DE	
Dingerdissen, Uwe	Seeheim-Jugenheim		DE	
Kuhlein, Klaus	Kelkheim		DE	
Manz, Andreas	Sinzheim		DE	
Fischer, Roland	Neckargemund		DE	

APPL-NO: 10/ 395752

DATE FILED: March 24, 2003

RELATED-US-APPL-DATA:

child 10395752 A1 20030324

parent division-of 09485804 20000428 US GRANTED

parent-patent 6603038 US

child 09485804 20000428 US

parent a-371-of-international PCT/EP98/04819 19980801 WO UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DE	197 34 974.9	1997DE-197 34 974.9	August 13, 1997

US-CL-CURRENT: 560/241, 502/325 , 502/344

ABSTRACT:

The invention relates to a method for producing a catalyst containing one or several metals from the group of metals comprising the subgroups Ib and VIIIb of the periodic table on porous support particles, characterised by a first step in which one or several precursors from the group of compounds of metals from sub-groups Ib and VIIIb of the periodic table is or are applied to a porous support, and a second step in which the porous, preferably nanoporous

support to which at least one precursor has been applied is treated with at least one reduction agent, to obtain the metal nanoparticles produced in situ in the pores of said support.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (131):

[0130] Some preferred catalyst systems which can be produced according to the invention, preferably coated catalysts, comprise, for example, not only palladium and gold but also potassium **acetate as activator and/or cadmium or barium compounds as promoters**.

PGPUB-DOCUMENT-NUMBER: 20030175775

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030175775 A1

TITLE: Ligand for G-protein coupled receptor GPR43 and uses thereof

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
LePoul, Emmanuel	Bruxelles		BE	
Detheux, Michel	Mons		BE	
Brezillon, Stephane	Dzlbeek		BE	
Lannoy, Vincent	Liernu		BE	
Parmentier, Marc	Beersel		BE	

APPL-NO: 10/ 337992

DATE FILED: January 7, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60346396 20020107 US

US-CL-CURRENT: 435/6, 435/7.1 , 514/1

ABSTRACT:

The present invention is related to the G-protein coupled orphan receptor GPR43 and the identification of short chain fatty acids as natural ligands of the receptor. The invention further relates to assays for the identification of agents that modulate GPR43 ligand binding and signalling activity, as well as compositions consisting essentially of an isolated GPR43 polypeptide and an isolated short chain fatty acid. The invention also relates to diagnostic methods and kits that take advantage of the novel interaction of GPR43 with short chain fatty acids.

PRIORITY

[0001] This application claims priority under 35 U.S.C. .sctn.119(e) to U.S. Provisional Application No. 60/346,396, filed Jan. 7, 2002.

---------- KWIC ---------

Detail Description Paragraph - DETX (108):
[0241] Additional examples of transcriptional control elements that are

responsive to changes in GPCR activity include, but are not limited to those responsive to the AP-1 transcription factor and those responsive to NF-.kappa.B activity. The consensus AP-1 binding site is the palindrome TGA(C/G)TCA (Lee et al., 1987, Nature 325: 368-372; Lee et al., 1987, Cell 49: 741-752). The AP-1 site is also responsible for mediating **induction by tumor promoters such as the phorbol ester 12-O-tetradecanoylphorbol- -acetate** (TPA), and are therefore sometimes also referred to as a TRE, for TPA-response element. AP-1 activates numerous genes that are involved in the early response of cells to growth stimuli. Examples of AP-1-responsive genes include, but are not limited to the genes for Fos and Jun (which proteins themselves make up AP-1 activity), Fos-related antigens (Fra) 1 and 2, I.kappa.B.alpha., ornithine decarboxylase, and annexins I and II.

PGPUB-DOCUMENT-NUMBER: 20030170856

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030170856 A1

TITLE: Regulation of human map kinase phosphatase-like enzyme

PUBLICATION-DATE: September 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Liou, Jiing-Ren	Belmont	MA	US	

APPL-NO: 10/ 363676

DATE FILED: April 24, 2003

PCT-DATA:
APPL-NO: PCT/EP01/09848
DATE-FILED: Aug 27, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/196, 435/194 , 435/320.1 , 435/325 , 435/6 , 435/69.1 , 536/23.2

ABSTRACT:

Reagents which regulate human MAP kinase phosphatase-like enzyme and reagents which bind to human MAP kinase phosphatase-like enzyme gene products can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, allergies including asthma, CNS disorders, diabetes, obesity, chronic obstructive pulmonary disease, cancer, and cardiovascular diseases.

---------- KWIC ---------

Detail Description Paragraph - DETX (295):
[0348] ERK activity is reduced in MAP kinase phosphatase-like enzyme overexpressing cells. Growth factor, hormone, and phorbol ester stimulation of PC12 cells have been known to activate the MAP kinase pathway and to stimulate the enzymatic activity of ERK-1 (61, 62, 65, 69, 71). The enzymatic activity of ERK-1 in wild type cells is compared with that in MAP kinase phosphatase-like enzyme overexpressing cells treated with these agents. PC12 cells treated for 10 minutes with mitogenic agents such as EGF, differentiating

agents such as NGF and forskolin, and the tumor **promoter phorbol 12-myristate 132-acetate (PMA), produce a robust activation** of ERK-1 as measured by an immune complex activity assay. MAP kinase phosphatase-like enzyme overexpressing clones are then treated with the same agents for the identical times. These clones show a dramatic reduction in the ability of growth factors and hormones to activate ERK-1. Quantitation of the immune complex assays shows that modest overexpression of MAP kinase phosphatase-like enzyme in PC12 cells inhibits growth factor- and hormone-induced activation of ERKs 80-90% cells compared to the fold activation seen in wild type PC12 cells. The basal ERK activity also appears to be lower in these overexpression cells as compared to wild type cells.

PGPUB-DOCUMENT-NUMBER: 20030158387

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030158387 A1

TITLE: Processed human chemokines PHC-1 and PHC-2

PUBLICATION-DATE: August 21, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Forssman, Wolf-Georg	Hannover		DE	
Detheux, Michel	Mons		BE	
Parmentier, Marc	Beersel		BE	
Standker, Ludger	Hannover		DE	
Kirchhoff, Frank	Ulm		DE	

APPL-NO: 10/ 202986

DATE FILED: July 24, 2002

RELATED-US-APPL-DATA:

child 10202986 A1 20020724

parent continuation-in-part-of 09891871 20010622 US PENDING

child 09891871 20010622 US

parent continuation-of PCT/BE00/00128 20001025 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DE	DE/19951336.8	1999DE-DE/19951336.8	October 25, 1999
EP	EP/00870140.1	2000EP-EP/00870140.1	June 22, 2000

US-CL-CURRENT: 530/351, 424/85.1 , 435/320.1 , 435/325 , 435/69.5 , 536/23.5

ABSTRACT:

The present invention is related to newly identified compounds, polynucleotide sequences encoding the amino acid sequences of the compounds, as well as agonists, antagonists or inhibitors of the compounds for chemokine receptors, especially the CCR-5 receptor and their use in the field of diagnostics and therapeutics involving the chemokine receptors.

PRIORITY

[0001] This application is a continuation in part of U.S. application Ser. No. 09/891,871, filed Jun. 22, 2001, which is a continuation of

PCT/BE00/00128, filed Oct. 25, 2000, which claims priority to EP00870140.1, filed Jun. 22, 2000, and DE 19951336.8, filed Oct. 25, 1999. The contents of each of these documents is incorporated herein in their entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (88):

[0244] Additional examples of transcriptional control elements that are responsive to changes in chemokine receptor activity include, but are not limited to those responsive to the AP-1 transcription factor and those responsive to NF-.kappa.B activity. The consensus AP-1 binding site is the palindrome TGA(C/G)TCA (Lee et al., 1987, Nature 325: 368-372; Lee et al., 1987, Cell 49: 741-752). The AP-1 site is also responsible for mediating **induction by tumor promoters such as the phorbol ester 12-O-tetradecanoylphorbol-.beta.-acetate** (TPA), and are therefore sometimes also referred to as a TRE, for TPA-response element. AP-1 activates numerous genes that are involved in the early response of cells to growth stimuli. Examples of AP-1-responsive genes include, but are not limited to the genes for Fos and Jun (which proteins themselves make up AP-1 activity), Fos-related antigens (Fra) 1 and 2, I.kappa.B.alpha.o, ornithine decarboxylase, and annexins I and II.

US-PAT-NO: 6610516

DOCUMENT-IDENTIFIER: US 6610516 B1

TITLE: Cell culture process

DATE-ISSUED: August 26, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Andersen; Dana C.	Redwood City	CA	N/A	N/A
Bridges; Tiffany M.	Burlingame	CA	N/A	N/A
Gawlitzek; Martin	Foster City	CA	N/A	N/A
Hoy; Cynthia A.	Hillsborough	CA	N/A	N/A

APPL-NO: 09/ 723545

DATE FILED: November 27, 2000

PARENT-CASE:

This is a divisional application claiming priority to application Ser. No. 09/553,924, filed Apr. 21, 2000, which claims priority to U.S. Provisional Application Serial No. 60/131,076, filed Apr. 26, 1999, the entire disclosure of which is hereby incorporated by reference.

US-CL-CURRENT: 435/70.1, 435/252.3 , 435/358 , 435/69.1 , 530/395

ABSTRACT:

A glycoprotein is produced by a process comprising culturing mammalian host cells expressing nucleic acid encoding a glycoprotein in the presence of (a) a factor that modifies growth state in a cell culture, (b) a divalent metal cation that can adopt and prefers an octahedral coordination geometry, and/or (c) a plasma component. In this process, the occupancy of an N-linked glycosylation site occupied only in a fraction of a glycoprotein is enhanced. Such culturing is preferably carried out at a temperature of between about 30.degree. C. and 35.degree. C. and/or in the presence of up to about 2 mM of a butyrate salt and/or in the presence of a cell-cycle inhibitor.

1 Claims, 16 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Detailed Description Text - DETX (16):

By "plasma component" is meant a constituent of normal plasma. This would include growth **promoters and tumor-promoting agents for endothelial cell growth, regulators of differentiation of epithelial tissues, glucagon, heparin, phorbol myristate acetate,** PRL, thyroglobulin, 8Br-cAMP, thrombin, vitamin A and its derivatives (retinoids such as retinoic acid, e.g., beta-all-trans retinoic acid), glutathione, steroids such as corticosterone, cortisol, and corticoids, e.g., glucocorticoids such as hydrocortisone, and hormones, preferably those that are vital hormones of metabolism such as estrogen, insulin, and thyroid hormones, e.g., thyroxine and tri-iodothyronine (T.sub.3). The thyroid hormones are preferred, and most preferably thyroxine and tri-iodothyronine. Since some serum, including fetal calf serum, contains thyroid hormones and the thyroid hormone binding protein at nanomolar levels, it is preferred to use serum-free medium, particularly if thyroid hormones are employed to enhance site-occupancy.

US-PAT-NO: 6603038

DOCUMENT-IDENTIFIER: US 6603038 B1

TITLE: Method for producing catalysts containing metal nanoparticles on a porous support, especially for gas phase oxidation of ethylene and acetic acid to form vinyl acetate

DATE-ISSUED: August 5, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hagemeyer; Alfred	Frankfurt	N/A	N/A	DE
Dingerdissen; Uwe	Seeheim-Jugenheim	N/A	N/A	DE
Kuhlein; Klaus	Kelkheim	N/A	N/A	DE
Manz; Andreas	Sinzheim	N/A	N/A	DE
Fischer; Roland	Neckargemund	N/A	N/A	DE

APPL-NO: 09/ 485804

DATE FILED: April 28, 2000

PARENT-CASE:

This application is a 371 of PCT/EP98/04819 filed Aug. 1, 1998.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	197 34 974	August 13, 1997

PCT-DATA:
APPL-NO: PCT/EP98/04819
DATE-FILED: August 1, 1998
PUB-NO: WO99/08791
PUB-DATE: Feb 25, 1999
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 560/241.1, 502/325 , 502/330

ABSTRACT:

The invention relates to a method for producing a catalyst containing one or several metals from the group of metals comprising the sub-groups Ib and VIIIb of the periodic table on porous support particles, characterized by a first step in which one or several precursors from the group of compounds of metals from sub-groups Ib and VIIIb of the periodic table is or are applied to a porous support, and a second step in which the porous, preferably nanoporous support to which at least one precursor has been applied is treated with at

least one reduction agent, to obtain the metal nanoparticles produced in situ in the pores of said support.

27 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ----------

Brief Summary Text - BSTX (113):
Some preferred catalyst systems which can be produced according to the invention, preferably coated catalysts, comprise, for example, not only palladium and gold but also potassium **acetate as activator and/or cadmium or barium compounds as promoters**.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	7	glnap$8	USPAT; US-PGPUB	2003/10/09 15:58
2	L2	347110	acetyl adj phosphate or acetylphosphate or acetate	USPAT; US-PGPUB	2003/10/09 15:59
3	L3	83953	promoter$1	USPAT; US-PGPUB	2003/10/09 15:59
4	L4	1448552	induc$8 or regulat$8 or activat$8 or modulat$8	USPAT; US-PGPUB	2003/10/09 15:59
5	L5	66	2 near10 4 near10 3	USPAT; US-PGPUB	2003/10/09 15:59
6	L6	10013	pps or phosphoenol adj pyruvate adj synthase$1	USPAT; US-PGPUB	2003/10/09 16:02
7	L7	98	6 near4 (gene$1 or sequence$1)	USPAT; US-PGPUB	2003/10/09 16:03
8	L8	24	7 same (overexpress$ or amplif$10 or increas$8)	USPAT; US-PGPUB	2003/10/09 16:04

PGPUB-DOCUMENT-NUMBER: 20030140368

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030140368 A1

TITLE: Plant defensins

PUBLICATION-DATE: July 24, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Famodu, Omolayo O.	Newark	DE	US	
Herrmann, Rafael	Wilmington	DE	US	
Lu, Albert L.	Newark	DE	US	
McCutchen, Billy Fred	Clive	IA	US	
Miao, Guo-Hua	Johnston	IA	US	
Presnail, James K.	Avondale	PA	US	
Rafalski, Jan Antoni	Wilmington	DE	US	
Weng, Zude	Des Plaines	IL	US	

APPL-NO: 10/ 178449

DATE FILED: June 21, 2002

RELATED-US-APPL-DATA:

child 10178449 A1 20020621

parent continuation-in-part-of 10030516 20011025 US PENDING

US-CL-CURRENT: 800/279, 435/184 , 435/320.1 , 435/419 , 435/69.1 , 530/370 , 536/23.6

ABSTRACT:

This invention relates to isolated nucleic acids encoding plant defensins. The invention also relates to the construction of a chimeric gene encoding all or a portion of the plant defensin, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of plant defensins in a transformed host cell.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This is a continuation-in-part of U.S. application Ser. No. 10/030,516 filed May 3, 2000; which claims the benefit of PCT Application PCT/US00/11952, filed May 3, 2000, and U.S. Provisional Application No. 60/133,039, filed May 7, 1999, the contents of which are herein incorporated by reference in their entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (57):

[0229] The nucleotide **sequence encoding the mature peptide of Pps**-AMP1 is set forth in SEQ ID NO:34. The nucleotide sequence (SEQ ID NO: 34) was PCR **amplified** from its corresponding cDNA clone, pps.pk0010.g2 (see Table 1). The 5' PCR primer incorporated an extra ATG sequence corresponding to a methionine residue immediately upstream of the mature peptide coding sequence for expression in bacteria. The 5' and 3' PCR primers were also designed to incorporate an NdeI and BamHI site, respectively, to facilitate cloning into the expression plasmid pET12a (Novagen, Madison Wis.). The resulting PCR product was TOPO-cloned into pCR2.1 (Invitrogen, Carlsbad, Calif.) and sequence verified. A NdeI-BamHI fragment containing the **Pps-AMP1 nucleotide sequence corresponding to the mature Pps**-AMP1 peptide, with the added methionine residue, was subcloned from pCR2.1 into the corresponding sites of pET12a placing the **Pps-AMP1 nucleotide sequence** encoding for the mature peptide under control of the T7 promoter. The pET12a-PpsAMP1 construct was transformed into a compatible expression host, BL21 (DE3, pLysS) (Invitrogen) or Origami (DE3, pLysS) (Novagen) and expression of the mature Pps-AMP1 peptide was induced by addition of IPTG as described in Example 6.

Detail Description Paragraph - DETX (99):

[0259] The presence of the nucleotide **sequence encoding the Pps**-AMP1 mature peptide was confirmed in transgenic soybean events by PCR **amplification**. Genomic DNA was prepared from callus by shaking approximately 100 .mu.l of callus at 1500 strokes/minute for 45 seconds in the Geno/Grinder in the presence of 1 steel ball (5/32"), 300 .mu.l of urea extraction buffer and 300 .mu.l of phenol/chloroform/isoamyl alcohol (25:24:1). Tubes were centrifuged at full speed for 5 minutes and 200 .mu.l of the aqueous phase transferred to a 96 deep well block. DNA was precipitated with an equal volume of isopropanol, centrifuged at full speed for 10 minutes, and the DNA pellets washed with 70% ethanol. After a further 5 minute centrifugation the supernatant was removed completely and the pellets dried in a speed vacuum. The DNA was resuspended in 100 .mu.l of 10 mM Tris HCL pH 8 and 2 .mu.l used for PCR **amplification**.

PGPUB-DOCUMENT-NUMBER: 20030134392

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030134392 A1

TITLE: Microorganisms and methods for overproduction of DAHP by cloned Pps gene

PUBLICATION-DATE: July 17, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Liao, James C.	Los Angeles	CA	US	

APPL-NO: 10/ 289788

DATE FILED: November 7, 2002

RELATED-US-APPL-DATA:

child 10289788 A1 20021107

parent division-of 09440503 19991115 US GRANTED

parent-patent 6489100 US

US-CL-CURRENT: 435/74, 435/252.33 , 435/320.1

ABSTRACT:

Genetic elements comprising expression vectors and a **gene coding for phosphoenol pyruvate synthase** is utilized to enhance diversion of carbon resources into the common aromatic pathway and pathways branching therefrom. The **overexpression** of phosphoenol pyruvate synthase **increases** DAHP production to near theoretical yields.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

Genetic elements comprising expression vectors and a **gene coding for phosphoenol pyruvate synthase** is utilized to enhance diversion of carbon resources into the common aromatic pathway and pathways branching therefrom. The **overexpression** of phosphoenol pyruvate synthase **increases** DAHP production to near theoretical yields.

Summary of Invention Paragraph - BSTX (18):

[0016] The present invention provides genetically engineered strains of

microorganisms that **overexpress the pps gene for increasing** the production of DAHP to near theoretical yields. The present invention also provides genetically engineered strains of microorganisms where at least one of the plasmids pPS341, pPSL706, pPS706, or derivatives thereof is transformed into a microorganism for **increasing** the production of DAHP to near theoretical yields.

Summary of Invention Paragraph - BSTX (19):

[0017] The present invention further provides a method for **increasing** carbon flow for the biosynthesis of DAHP in a host cell comprising the steps of transforming into the host cell recombinant DNA comprising a **pps gene so that Pps** is expressed at enhanced levels relative to wild type host cells, concentrating the transformed cells through centrifugation, resuspending the cells in a minimal, nutrient lean medium, fermenting the resuspended cells, and isolating DAHP from the medium.

Summary of Invention Paragraph - BSTX (20):

[0018] The present invention further provides methods of **increasing** carbon flow into the common aromatic pathway of a host cell comprising the step of transforming the host cell with recombinant DNA comprising a **pps gene so that Pps** is expressed at appropriate point in the metabolic pathways at enhanced levels relative to wild type host cells.

Detail Description Paragraph - DETX (3):

[0031] The inventor have found that cell lines can be developed that **increase** the carbon flux into DAHP production and achieve near theoretical yields of DAHP by **overexpressing** phosphoenolpyruvate synthase (Pps) in the cell lines. **Overexpression** of Pps can **increase** the final concentration and yield of DAHP by as much as two-fold, to a near theoretical maximum as compared to wild type cell lines. The **overexpression** of Pps is achieved by transforming a cell line with recombinant DNA comprising a **pps gene so that Pps** is expressed at enhanced level relative to the wild type cell line and so that the yield of DAHP approaches its theoretical value.

Detail Description Paragraph - DETX (5):

[0033] Besides the use of the **pps gene,** the present invention also provides for transfer of genetic elements comprising the tkt gene, the gene coding for DAHP synthase (aroF in E. coli), the gene coding for 3-dehydroquinate synthase (aroB in E. coli), or other genes encoding enzymes that catalyze reactions in the common aromatic pathway. Such a cell transformation can be achieved by transferring one or more plasmids that contain genes that code for enzymes that **increase** the carbon flux for DAHP synthesis and for subsequent synthesis of other desired cyclic, pre-aromatic, and aromatic metabolites. As a result of this transfer of genetic element(s), more carbon enters and moves through the common aromatic pathway relative to wild type host cells not containing the genetic elements of the present invention.

Detail Description Paragraph - DETX (6):

[0034] In one embodiment, the present invention comprises a method for **increasing** carbon flow into the common aromatic pathway of a host cell by **increasing** the production of DAHP through the **overexpression** of Pps at the appropriate point in the common aromatic pathway to provide additional PEP at the point where PEP condenses with E4P. **Increasing** carbon flow requires the step of transforming the host cell with recombinant DNA containing a **pps gene so that Pps is overexpressed** at enhanced levels relative to wild type host cells. DAHP is then produced by fermentation of the transformed cell in a nutrient medium where the DAHP can be extracted from the medium on a batch wise or continuous extraction procedure.

Detail Description Paragraph - DETX (7):

[0035] In another embodiment, the present invention involves the co-**overexpression of a pps gene** and other genes coding for enzymes of the common aromatic pathway where additional genetic material is transformed into the host cell. The genes transferred can include the tkt gene, DAHP synthase gene and DHQ synthase gene (preferably the aroF or aroB genes, respectively). Although the work so far has centered around transforming certain host cell strains of E. coli such as AB2847 aroB, this particular host cell may not be the preferred host cells for the commercial production of DAHP or DAHP metabolites through the **overexpression** of Pps.

Detail Description Paragraph - DETX (8):

[0036] Another embodiment of the present invention is a method for enhancing a host cell's biosynthetic production of compounds derived from the common aromatic pathway. This method involves the step of **increasing** expression of Pps in the host cell relative to a wild type host cell. The step of **increasing** expression of Pps can include transferring into the host cell a vector carrying the **pps gene**. The **overexpression** of Pps results in forcing **increased** carbon flow into the biosynthesis of DAHP.

Detail Description Paragraph - DETX (9):

[0037] In another embodiment of the present invention, a method for enhancing a host cell's biosynthetic production of compounds derived from the common aromatic pathway relative to wild type host cell's biosynthetic production of such compound is provided. This method requires the step of **increasing** expression in a host cell of a protein catalyzing conversion of pyruvate to PEP. The expression of such a protein can involve transferring into the host cell recombinant DNA including a **pps gene**.

Detail Description Paragraph - DETX (56):

[0083] One preferred embodiment of the present invention encompasses modification of a host cell to cause **overexpression** of an enzyme having the catalytic properties of naturally derived Pps, and, thereby maximizing the yield of DAHP to near theoretical yields. Enzymes having the catalytic activity of Pps include, but are not limited to, Pps produced by expression in whole cells of a naturally derived **pps gene,** enzymes produced by expression in whole cells of a naturally derived **pps gene** modified by sequence deletion or

addition so that the expressed enzyme has an amino acid **sequence that varies from unmodified Pps,** abzymes produced to have catalytic sites with steric and electronic properties corresponding to catalytic sites of Pps, or other proteins produced to have the capability of catalyzing the conversion of pyruvate to PEP by any other art recognized means.

Detail Description Paragraph - DETX (58):

[0085] Additionally, the transformation of DNA, including the **pps gene,** into microorganisms engineered for the **overexpression** of other substrates, and/or **overexpression** or derepression of enzymes in the pentose phosphate or common aromatic pathway can be used to tailor the microorganism to achieve near theoretical yields of such DAHP metabolites as tyrosine, tryptophan, phenylalanine, and other aromatic metabolites such as indigo, catechol and quinoid organics such as quinic acid, benzoquinone, and hydroquinone.

Detail Description Paragraph - DETX (93):

[0119] This example demonstrates that the E. Coli AB2847 is unable to utilize DAHP, and accumulates DAHP in the medium if DAHP synthase is **overexpressed**. This strain was used as a host for detecting the flux committed to the aromatic pathways. Since Draths et al. (Draths, K. M., D. L. Pompliano, D. L. Conley, J. W. Frost, A. Berry, G. L. Disbrow, R. J. Staversky, and J. C. Lievense, "Biocatalytic synthesis of aromatics from D-glucose: The role of transketolase," J. Am. Chem. Soc., 1992, 114, 3956-3962) have shown a possible limitation in the production of DAHP by E4P, pATI (containing both aroG.sup.fbr and tktA) was transformed into AB2847 to eliminate the limitation of E4P. To test whether PEP supply limits DAHP production, PEP synthase (Pps) was **overexpressed** in AB2847/pAT1 by transforming plasmid pPS341 into this strain. 20-70 copies of the **pps gene** were expressed in the host cells. As a control, pPS341 was substituted by pPS341X1, which encodes an inactive, but stable **pps gene** product. The use of the inactive Pps control allowed discrimination between the effect of Pps activity and that of protein **overexpression**. AB2847/pAT1/pUHE23-2 and AB2847/pAT1 were also used without any other plasmid as additional controls to identify the effect of the cloning vector, pUHE23-2, on DAHP production.

Detail Description Paragraph - DETX (98):

[0123] As shown above, Pps **overexpression** improved DAHP production from glucose. We were interested to know whether the basal level of Pps expression in glucose medium contributed to the production of DAHP. Therefore, the chromosomal **pps gene** in strain AB2847 was knocked out. The resulting strain (JCL1362) was used as the host to repeat the above experiments. Results show that inactivation of chromosomal pps did not significantly affect the DAHP production in strains containing pRW5 or pATI (FIG. 2B). Therefore, the basal level of pps expression in glucose medium did not contribute to the DAHP production.

Detail Description Paragraph - DETX (117):

[0137] Quinoid organics can be derived from dehydroquinate which is a

down-stream metabolite of DAHP. To produce quinic acid, E. coli AB2848 aroD harboring pTW8090A which contains the gene qad (quinic acid dehydrogenase from Klebsiella pneumoniae) (ref: Draths, Ward, and Frost, 1992, JACS, 114, 9725-9726), and pKD136 (ref: same as above) which contains tkt, aroF, and aroB genes can be used as a host. The **pps gene** can be cloned into one of these plasmids and be simultaneously **overexpressed**. It has been reported that at least 80 mM of D-glucose can be converted into 25 mM of quinic acid. After cell removal, quinic acid in the supernatant can be converted into benzoquinone after addition of sulfuric acid and technical grade manganese (IV) dioxide and heating at 100.degree. C. for 1 h. In the absence of acidification, aqueous solutions of purified quinic acid were converted to hydroquinone in 10% yield upon heating at 100.degree. C. for 18 h with technical grade manganese dioxide.

Claims Text - CLTX (11):
10. A process for the production of DAHP which comprises cultivating a microorganism in a nutrient medium and **overexpressing the Pps gene**.

Claims Text - CLTX (12):
11. The process of claim 10 wherein the step of **overexpressing the Pps gene** comprises transforming a plasmid selected from the group consisting of pPS341, pPSL706, and pPS706 the microorganism.

Claims Text - CLTX (15):
14. A culture containing a microorganism modified with DNA comprising a **pps gene,** said culture being capable of producing **increased** levels of DAHP upon fermentation in an aqueous nutrient medium containing assimilable sources of carbon, nitrogen and inorganic substances.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	7	glnap$8	USPAT; US-PGPUB	2003/10/09 15:58
2	L2	347110	acetyl adj phosphate or acetylphosphate or acetate	USPAT; US-PGPUB	2003/10/09 15:59
3	L3	83953	promoter$1	USPAT; US-PGPUB	2003/10/09 15:59
4	L4	1448552	induc$8 or regulat$8 or activat$8 or modulat$8	USPAT; US-PGPUB	2003/10/09 15:59
5	L5	66	2 near10 4 near10 3	USPAT; US-PGPUB	2003/10/09 15:59
6	L6	10013	pps or phosphoenol adj pyruvate adj synthase$1	USPAT; US-PGPUB	2003/10/09 16:02
7	L7	98	6 near4 (gene$1 or sequence$1)	USPAT; US-PGPUB	2003/10/09 16:03
8	L8	24	7 same (overexpress$ or amplif$10 or increas$8)	USPAT; US-PGPUB	2003/10/09 16:04
9	L9	21	7 and 2	USPAT; US-PGPUB	2003/10/09 16:08

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DOCUMENT-IDENTIFIER: US 20030165575 A1

TITLE: Methods and compositions for treatment and diagnosis of alzheimer disease and other disorders

PUBLICATION-DATE: September 4, 2003

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child 10218182 A1 20020812

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child 08743342 19961101 US

parent continuation-of 08182817 19940113 US ABANDONED

US-CL-CURRENT: 424/639, 424/682 , 514/2

ABSTRACT:

The present invention is directed to methods for treating Alzheimer disease and other disorders associated with the presence of neurofibrillary tangles (NFTs) by increasing the activity of a phosphatase towards abnormal hyperphosphorylated tau ("AD P-tau") present in the NFTs of paired helical filaments in the neurons of patients having Alzheimer disease or other NFT-associated disorder. Pharmaceutical compositions and diagnostic methods are also provided. The inventions provide methods of treatment by administering to a subject a therapeutically effective amount of a composition comprising a molecule which increases protein phosphatase activity toward AD P-tau, a phosphatase which dephosphorylates AD P-tau, or a nucleic acid encoding such a phosphatase.

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Detail Description Paragraph - DETX (11):

[0056] In a specific embodiment, the molecule increases the activity towards AD P-tau of at least one of the foregoing PPs' and does not inhibit any such activity of the foregoing PPs. In another specific embodiment, the molecule is not Mg.sup.2+. The metal can be in ionic form, salt form, or conjugate. The manganese is preferably in the form of a water-soluable salt such as but not limited to manganese chloride, manganese sulfate, manganese **acetate,** manganese gluconate, manganese lactate, and manganese citrate. The manganese may also be in the form of other compounds such as manganese hypophosphite, manganese silicate, manganese sulfide, manganese iodide, manganese phosphate, manganese borate, manganese bromide, manganese oleate, manganese nitrate, manganese carbonate, manganese carbonyl, manganese difluoride, manganese trifluoride, manganese oxalate, manganese oxide, manganese dioxide, manganese selenide, manganese sesquioxide, etc. In a specific embodiment, the manganese is not in the form of manganese pyruvate or a manganese chelate of an alkylamino-ester of phosphoric acid (e.g., manganese aminoethyl phosphate). Preferably, such molecules are administered orally, although any form of administration known in the art can be used (see Section 5.5 infra).

Detail Description Paragraph - DETX (18):

[0062] In another embodiment, nucleic acids encoding one or more of the aforesaid PPs can be administered in vivo such that the encoded PP is expressed for therapeutic effect. The cloning and/or nucleotide **sequences of PPs** are available in the art, e.g., as described in the following publications. For PP-1: Sasaki et al, 1990, Jpn. J. Cancer Res. 81: 1272-1280. For PP-1.alpha.: Berndt et al., 1987, FEBS Lett. 223:340-346. For PP-2A: Kitagawa et al., 1988, Biochim. Biophys. Acta 951:123-129; Kitagawa et al., 1988, Biochem. Biophys. Res. Commun. 157:821-827; Sasaki et al., 1990, Biochem. Biophys. Res. Commun. 170:169-175. For PP-2B: Muramatsu and Kincaid, 1993, Biochim. Biophys. Acta 1178:117-120; Guerini and Klee, 1989, Proc. Natl. Acad. Sci. USA 86:9183-9187; Kincaid et al., 1990, J. Biol. Chem. 265:11312-11319; Kincaid et al, 1988, Proc. Natl. Acad. Sci. USA 85:8983-8987.

Detail Description Paragraph - DETX (127):

[0154] Isolation of AD P-tau and Acid-soluble Tau. AD P-tau was isolated by the method of Kopke et al. (1993, J. Biol. Chem. 268:24374-24384). For acid-soluble tau AD and control brains were homogenized with an Omnimixer at 4.degree. C. in 2% perchloric acid (10 ml/g of tissue) containing protease inhibitors, as described previously (Kopke et al., 1993, J. Biol. Chem. 268:24374-24384). The homogenates were spun at 100,000.times.g for 30 min. The supernatant was brought to 2.5% perchloric acid and centrifuged again for another 30 min. The supernatant was concentrated approximately 10 times by Amicon filtration and dialyzed against 20 mM sodium **acetate** buffer, pH 5.6. After dialysis, the extract was spun for 10 min at 100,000.times.g, and the supernatant was subjected to carboxyl methyl chromatography using Millipore Mem Sep CM 1010 disk (Millipore, Bedford, Mass.). The protein sample (25-40 mg/50 ml) was loaded at a flow rate of 0.5 ml/min, and tau was eluted with 0.25 M

NaCl in 20 mM sodium **acetate** buffer, pH 5.6. The eluate was analyzed by absorbance at 254 nm and immunoslot blot using antiserum 92e to tau. The tau peak was pooled and dialyzed against 5 mM MES buffer, pH 6.7, containing 0.05 mM EGTA. Aliquots of approximately 500 .mu.l, containing 120 .mu.g of protein, were dried in a Speed Vac concentrator (Savant, Farmingdale, N.Y.). For each assay, the lyophilized tau preparation was reconstituted in [fraction (1/10)] vol. water immediately before use.

Claims Text - CLTX (8):

7. The method according to claim 4 in which the molecule is selected from the group consisting of manganese chloride, manganese sulfate, manganese **acetate,** manganese gluconate, manganese lactate and manganese citrate.

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TITLE: Plant defensins

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INVENTOR-INFORMATION:

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APPL-NO: 10/ 178449

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US-CL-CURRENT: 800/279, 435/184 , 435/320.1 , 435/419 , 435/69.1 , 530/370 , 536/23.6

ABSTRACT:

This invention relates to isolated nucleic acids encoding plant defensins. The invention also relates to the construction of a chimeric gene encoding all or a portion of the plant defensin, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of plant defensins in a transformed host cell.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This is a continuation-in-part of U.S. application Ser. No. 10/030,516 filed May 3, 2000; which claims the benefit of PCT Application PCT/US00/11952, filed May 3, 2000, and U.S. Provisional Application No. 60/133,039, filed May 7, 1999, the contents of which are herein incorporated by reference in their entirety.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (6):
[0025] FIG. 4 depicts the results obtained from SCN assays on soybean plants transformed with a vector comprising the IFS1 promoter, the BAA signal **sequence, and the mature peptide region of Pps**-AMP1. Average number of cysts for the Jack soybean cultivar representing T0 transformants that did not contain the heterologous DNA (Neg control); the Essex soybean variety (which is susceptible to SCN); and selected PCR positive T0 IFS1:BAA-mature PpsAMP1 transformants. Measurements are based on 3 plants/event.

Brief Description of Drawings Paragraph - DRTX (7):
[0026] FIG. 5 depicts the results obtained from Sclerotinia sclerotiorum leaf assays on soybean plants transformed with a vector comprising the UCP1 promoter, the BAA signal **sequence and the mature peptide region of Pps**-AMP1. Average lesion size for Essex soybean leaves (a variety susceptible to SCN); Jack soybean cultivar leaves representing T0 transformants that did not contain the heterologous DNA (Neg control); and selected transgenic UCP1:BAA-mature Pps-AMP1 T0 plants. Measurements are based on 3 plants/event, 2 leaves/plant. Two measurements were made across the widest tranects of the lesion and then multiplied to give an approximate surfact area for thelesion.

Brief Description of Drawings Paragraph - DRTX (8):
[0027] FIG. 6 depicts the results obtained from Sclerotinia sclerotiorum leaf assays on soybean plants transformed with a vector comprising the IFS1 promoter, the BAA signal **sequence, and the mature peptide region of Pps**-AMP1. Average lesion size for control Essex soybean leaves and selected transgenic IFS1:BAA-mature PpsAMP1 T0 plants. Measurements are based on 3 plants/event, 2 leaves/plant. Two measurements were made across the widest transects of the lesion and then multiplied to give an approximate surface area for the lesion.

Brief Description of Drawings Paragraph - DRTX (156):
[0174] Suitable surface-active agents include, but are not limited to, anionic compounds such as a carboxylate of, for example, a metal; a carboxylate of a long chain fatty acid; an N-acylsarcosinate; mono or di-esters of phosphoric acid with fatty alcohol ethoxylates or salts of such esters; fatty alcohol sulfates such as sodium dodecyl sulfate, sodium octadecyl sulfate, or sodium cetyl sulfate; ethoxylated fatty alcohol sulfates; ethoxylated alkylphenol sulfates; lignin sulfonates; petroleum sulfonates; alkyl aryl sulfonates such as alkyl-benzene sulfonates or lower alkylnaphtalene sulfonates, e.g., butyl-naphthalene sulfonate; salts of sulfonated naphthalene-formaldehyde condensates; salts of sulfonated phenol-formaldehyde condensates; more complex sulfonates such as the amide sulfonates, e.g., the sulfonated condensation product of oleic acid and N-methyl taurine; or the dialkyl sulfosuccinates, e.g., the sodium sulfonate or dioctyl succinate. Non-ionic agents include condensation products of fatty acid esters, fatty alcohols, fatty acid amides or fatty-alkyl- or alkenyl-substituted phenols with ethylene oxide, fatty esters of polyhydric alcohol ethers, e.g., sorbitan fatty acid esters, condensation products of such esters with ethylene oxide, e.g.

polyoxyethylene sorbitar fatty acid esters, block copolymers of ethylene oxide and propylene oxide, acetylenic glycols such as 2, 4, 7, 9-tetraethyl-5-decyn-4,7-diol, or ethoxylated acetylenic glycols. Examples of a cationic surface-active agent include, for instance, an aliphatic mono-, di-, or polyamine such as an **acetate,** naphthenate, or oleate; or oxygen-containing amine such as an amine oxide of polyoxyethylene alkylamine; an amide-linked amine prepared by the condensation of a carboxylic acid with a di- or polyamine; or a quaternary ammonium salt.

Brief Description of Drawings Paragraph - DRTX (168):

[0186] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl **acetate,** polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

Detail Description Paragraph - DETX (57):

[0229] The nucleotide **sequence encoding the mature peptide of Pps**-AMP1 is set forth in SEQ ID NO:34. The nucleotide sequence (SEQ ID NO: 34) was PCR amplified from its corresponding cDNA clone, pps.pk0010.g2 (see Table 1). The 5' PCR primer incorporated an extra ATG sequence corresponding to a methionine residue immediately upstream of the mature peptide coding sequence for expression in bacteria. The 5' and 3' PCR primers were also designed to incorporate an NdeI and BamHI site, respectively, to facilitate cloning into the expression plasmid pET12a (Novagen, Madison Wis.). The resulting PCR product was TOPO-cloned into pCR2.1 (Invitrogen, Carlsbad, Calif.) and sequence verified. A NdeI-BamHI fragment containing the **Pps-AMP1 nucleotide sequence corresponding to the mature Pps**-AMP1 peptide, with the added methionine residue, was subcloned from pCR2.1 into the corresponding sites of pET12a placing the **Pps-AMP1 nucleotide sequence** encoding for the mature peptide under control of the T7 promoter. The pET12a-PpsAMP1 construct was transformed into a compatible expression host, BL21 (DE3, pLysS) (Invitrogen) or Origami (DE3, pLysS) (Novagen) and expression of the mature Pps-AMP1 peptide was induced by addition of IPTG as described in Example 6.

Detail Description Paragraph - DETX (66):

[0234] The unfolded protein was purified by reverse phase chromatography on a Vydac.TM. C 18 column (10 micron particle, 300 Angstrom pore size, Part number 218TP101510, Grace Vydac, Calif.) using a two step gradient consisting of Solvent A (95% H.sub.2O, 5% acetonitrile, 0.1% trifluoroacetic acid) and Solvent B (5% H.sub.2O, 95% acetonitrile, 0.1% trifluoroacetic acid). The first step of the gradient was 10% to 24% Solvent B at a flow rate of 3 ml/min

for 3 min. The second step was from 24% to 40% Solvent B at a flow rate of 3 ml/min for 14 minutes. The protein was monitored by absorbance at 214 nm. Prior to loading on the column, the sample was adjusted to 1% trifluoroacetic acid and any precipitated material removed by centrifugation. The unfolded, reduced Pps-AMP1 mature peptide eluted at approximately 37% solvent B. Fractions corresponding to the unfolded peak of Pps-AMP1 mature protein were pooled and the protein concentration adjusted to 0.1 mg/mL-0.5 mg/mL by addition of 40% acetonitrile. The solution was brought to 0.1 M ammonium **acetate,** pH 6-9, and 1.0 mM reduced glutathione and stirred at room temperature until the Pps-AMP1 mature peptide was completely folded as determined by LC/MS analysis. Generally 24 hours was found to be sufficient for complete folding. Folded Pps-AMP1 mature peptide was purified by reverse phase chromatography on a Vydac.TM. C18 column (10 micron particle, 300 Angstrom pore size, Part number 218TP101510). The protein was eluted with a linear gradient (Solvent A-95% H2O, 5% acetonitrile and 0.1% trifluoroacetic acid, Solvent B--5% H2O, 95% acetonitrile, 0.1% trifluoroacetic acid) from 10% to 60% Solvent B in 45 minutes at 3 ml/min and monitored by absorbance at 214 run. Pure, folded Pps-AMP1 mature peptide was collected and freeze-dried. The freeze-dried protein can be resolubilized in water and a protein assay performed to determine concentration prior to bioassay.

Detail Description Paragraph - DETX (86):

[0250] A synthetic version of Pps-AMP1 mature peptide (SEQ ID NO:35) operably linked to a modified barley alpha amylase (BAA) signal peptide (SEQ ID NO: 50) (Rahmatullah R et al. (1989) Plant Mol. Biol. 12(1): 119-121) was constructed with a codon-bias representative of Glycine max (see SEQ ID NO: 36 for the synthetic nucleotide molecule comprising the BAA signal peptide operatively linked to the Pps-AMP1 mature peptide and SEQ ID NO: 37 for the protein sequence corresponding to SEQ ID NO: 36). Codon usage based on Glycine max was chosen to maximize the expression of Pps-AMP1 mature peptide in soybean plants. The codon preference selected for the Pps-AMP1 mature peptide as well as the BAA signal sequence was derived from the codon usage database available at Kazusa (available from www.Kazusa.orjp/codon/). See also Table 7. The BAA signal **sequence was added to the Pps**-AMP1 mature peptide coding **sequence to facilitate the export of Pps**-AMP1 mature peptide out of the cell and into the intercellular space.

Detail Description Paragraph - DETX (93):

[0255] A selectable marker expression cassette that can be used to facilitate soybean transformation comprises the .sup.35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) Nature 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from E. coli; Gritz et al. (1983) Gene 25:179-188), and the 3' region of the nopaline synthase (NOS) gene from the T-DNA of the Ti plasmid of Agrobacterium tumefaciens. The expression cassette comprising the nucleotide **sequence encoding the Pps**-AMP1 mature peptide operably linked to a promoter can be isolated as a restriction fragment. This fragment is then inserted into a unique restriction site of the vector carrying the marker gene.

Detail Description Paragraph - DETX (99):

[0259] The presence of the nucleotide **sequence encoding the Pps**-AMP1 mature peptide was confirmed in transgenic soybean events by PCR amplification. Genomic DNA was prepared from callus by shaking approximately 100 .mu.l of callus at 1500 strokes/minute for 45 seconds in the Geno/Grinder in the presence of 1 steel ball (5/32"), 300 .mu.l of urea extraction buffer and 300 .mu.l of phenol/chloroform/isoamyl alcohol (25:24:1). Tubes were centrifuged at full speed for 5 minutes and 200 .mu.l of the aqueous phase transferred to a 96 deep well block. DNA was precipitated with an equal volume of isopropanol, centrifuged at full speed for 10 minutes, and the DNA pellets washed with 70% ethanol. After a further 5 minute centrifugation the supernatant was removed completely and the pellets dried in a speed vacuum. The DNA was resuspended in 100 .mu.l of 10 mM Tris HCL pH 8 and 2 .mu.l used for PCR amplification.

Detail Description Paragraph - DETX (105):

[0263] Total RNA was extracted from a subset of IFS1:BAA-mature Pps-AMP1 events by collecting one leaflet per event into a 2 ml sterile screw cap tube, adding 2 steel balls ([fraction (5/32)]"), and 1.0 ml of Trizol Reagent (GIBCO-BRL). Leaflets were homogenized in a DNA FastPrep instrument at a speed of 4.5 for 45 seconds and the tubes centrifuged for 10 min at 4.degree. C. The supernatant was extracted with chloroform and the RNA precipitated from the aqueous phase with cold isopropyl alcohol. After a 10 minute centrifugation step the pellet was washed with 70% ethanol and dried in a speed vacuum. The pellet was resuspended in 90 .mu.l of DEPC-treated H.sub.2O and amplification grade DNAseI (1 U) and 10.times.DNAse I buffer added to a total volume of 100 .mu.l. The reaction was incubated at 37.degree. C. for 15 min, twice extracted with an equal volume of phenol/chloroform/isoamyl alcohol and the RNA precipitated from the aqueous phase with 0.1 volume 3 M sodium **acetate** and 2.5 volume of 100% ethanol. After centrifugation the pellet was resuspended in 30 .mu.l of DEPC-H.sub.2O. RNA samples were stored at -80.degree. C. until use.

Detail Description Paragraph - DETX (112):

[0268] Whole plant-bioassays of events containing the nucleotide **sequence encoding the mature Pps**-AMP1 peptide under control of the UCP1 (see FIG. 3) or IFS1 (see FIG. 4) promoters displayed a range of resistance (as determined by the number of cysts) relative to the negative control (transformed Jack soybean cultivar that did not contain heterologous DNA) and the wild-type Essex cultivar soybean plants. Results from a subset of plants tested are shown in FIGS. 3 and 4. The results of the full set of plants tested appears in Appendix 1 and 2. This range of resistance (based on cyst number) is expected for the population of events generated since differences in Pps-AMP1 mature peptide expression levels may be variable depending on such parameters as, integration site and copy number, among others. While there was a reduction in cyst numbers with both constructs compared to the negative control Jack cultivar or the Essex cultivar, in general, those events containing the Pps-AMP1 mature peptide encoding nucleotide sequence operatively linked to the IFS1 promoter (IFS1:BAA-mature Pps-AMP1) performed better than those events containing the Pps-AMP1 mature peptide encoding nucleotide sequence operatively linked to the UCP1 promoter (UCP1:BAA-mature Pps-AMP1). Several events exhibited excellent resistance with no cysts observed in the root systems.

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TITLE: Microorganisms and methods for overproduction of DAHP by cloned **Pps gene**

PUBLICATION-DATE: July 17, 2003

INVENTOR-INFORMATION:

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parent division-of 09440503 19991115 US GRANTED

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US-CL-CURRENT: 435/74, 435/252.33 , 435/320.1

ABSTRACT:

Genetic elements comprising expression vectors and a **gene coding for phosphoenol pyruvate synthase** is utilized to enhance diversion of carbon resources into the common aromatic pathway and pathways branching therefrom. The overexpression of phosphoenol pyruvate synthase increases DAHP production to near theoretical yields.

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Abstract Paragraph - ABTX (1):

Genetic elements comprising expression vectors and a **gene coding for phosphoenol pyruvate synthase** is utilized to enhance diversion of carbon resources into the common aromatic pathway and pathways branching therefrom. The overexpression of phosphoenol pyruvate synthase increases DAHP production to near theoretical yields.

Title - TTL (1):

Microorganisms and methods for overproduction of DAHP by cloned **Pps gene**

Summary of Invention Paragraph - BSTX (18):
[0016] The present invention provides genetically engineered strains of microorganisms that overexpress the **pps gene** for increasing the production of DAHP to near theoretical yields. The present invention also provides genetically engineered strains of microorganisms where at least one of the plasmids pPS341, pPSL706, pPS706, or derivatives thereof is transformed into a microorganism for increasing the production of DAHP to near theoretical yields.

Summary of Invention Paragraph - BSTX (19):
[0017] The present invention further provides a method for increasing carbon flow for the biosynthesis of DAHP in a host cell comprising the steps of transforming into the host cell recombinant DNA comprising a **pps gene so that Pps** is expressed at enhanced levels relative to wild type host cells, concentrating the transformed cells through centrifugation, resuspending the cells in a minimal, nutrient lean medium, fermenting the resuspended cells, and isolating DAHP from the medium.

Summary of Invention Paragraph - BSTX (20):
[0018] The present invention further provides methods of increasing carbon flow into the common aromatic pathway of a host cell comprising the step of transforming the host cell with recombinant DNA comprising a **pps gene so that Pps** is expressed at appropriate point in the metabolic pathways at enhanced levels relative to wild type host cells.

Summary of Invention Paragraph - BSTX (24):
[0022] The present invention further provides a genetic element comprising a **pps gene** and one or more genes selected from the group consisting of an aroF gene, aroG gene, aroH gene, and an aroB gene.

Summary of Invention Paragraph - BSTX (25):
[0023] The present invention further provides a DNA molecule comprising a vector carrying a **gene coding for Pps**.

Detail Description Paragraph - DETX (3):
[0031] The inventor have found that cell lines can be developed that increase the carbon flux into DAHP production and achieve near theoretical yields of DAHP by overexpressing phosphoenolpyruvate synthase (Pps) in the cell lines. Overexpression of Pps can increase the final concentration and yield of DAHP by as much as two-fold, to a near theoretical maximum as compared to wild type cell lines. The overexpression of Pps is achieved by transforming a cell line with recombinant DNA comprising a **pps gene so that Pps** is expressed at enhanced level relative to the wild type cell line and so that the yield of DAHP approaches its theoretical value.

Detail Description Paragraph - DETX (5):

[0033] Besides the use of the **pps gene,** the present invention also provides for transfer of genetic elements comprising the tkt gene, the gene coding for DAHP synthase (aroF in E. coli), the gene coding for 3-dehydroquinate synthase (aroB in E. coli), or other genes encoding enzymes that catalyze reactions in the common aromatic pathway. Such a cell transformation can be achieved by transferring one or more plasmids that contain genes that code for enzymes that increase the carbon flux for DAHP synthesis and for subsequent synthesis of other desired cyclic, pre-aromatic, and aromatic metabolites. As a result of this transfer of genetic element(s), more carbon enters and moves through the common aromatic pathway relative to wild type host cells not containing the genetic elements of the present invention.

Detail Description Paragraph - DETX (6):

[0034] In one embodiment, the present invention comprises a method for increasing carbon flow into the common aromatic pathway of a host cell by increasing the production of DAHP through the overexpression of Pps at the appropriate point in the common aromatic pathway to provide additional PEP at the point where PEP condenses with E4P. Increasing carbon flow requires the step of transforming the host cell with recombinant DNA containing a **pps gene so that Pps** is overexpressed at enhanced levels relative to wild type host cells. DAHP is then produced by fermentation of the transformed cell in a nutrient medium where the DAHP can be extracted from the medium on a batch wise or continuous extraction procedure.

Detail Description Paragraph - DETX (7):

[0035] In another embodiment, the present invention involves the co-overexpression of a **pps gene** and other genes coding for enzymes of the common aromatic pathway where additional genetic material is transformed into the host cell. The genes transferred can include the tkt gene, DAHP synthase gene and DHQ synthase gene (preferably the aroF or aroB genes, respectively). Although the work so far has centered around transforming certain host cell strains of E. coli such as AB2847 aroB, this particular host cell may not be the preferred host cells for the commercial production of DAHP or DAHP metabolites through the overexpression of Pps.

Detail Description Paragraph - DETX (8):

[0036] Another embodiment of the present invention is a method for enhancing a host cell's biosynthetic production of compounds derived from the common aromatic pathway. This method involves the step of increasing expression of Pps in the host cell relative to a wild type host cell. The step of increasing expression of Pps can include transferring into the host cell a vector carrying the **pps gene**. The overexpression of Pps results in forcing increased carbon flow into the biosynthesis of DAHP.

Detail Description Paragraph - DETX (9):

[0037] In another embodiment of the present invention, a method for enhancing a host cell's biosynthetic production of compounds derived from the common aromatic pathway relative to wild type host cell's biosynthetic production of such compound is provided. This method requires the step of

increasing expression in a host cell of a protein catalyzing conversion of pyruvate to PEP. The expression of such a protein can involve transferring into the host cell recombinant DNA including a **pps gene**.

Detail Description Paragraph - DETX (10):

[0038] In another preferred embodiment, the present invention comprises a genetic element comprising the **pps gene** and a gene selected from the group consisting of a aroF gene, a aroB gene, and a tkt gene. Such a genetic element can comprise plasmid pPS341, a vector carrying a **pps gene**.

Detail Description Paragraph - DETX (51):

[0078] In previous work, the inventor demonstrated that overexpression of Pps in host cells cultured on nutrient rich, glucose containing medium led to growth inhibition, increased glucose consumption, and excretion of pyruvate and **acetate**. Their previous study also showed that the effects of Pps overexpression on DAHP production, in actively growing cultures, are not as significant, and that the adverse effects of Pps overexpression on cell growth negated any beneficial effects on DAHP production.

Detail Description Paragraph - DETX (55):

[0082] PEP is also a precursor to the pathways that utilize the Ppc enzyme coded by the ppc gene. It has been reported that the deletion of ppc increased the production of phenylalanine and **acetate**. Moreover, it has been shown that the overexpression of Ppc in a wild-type host reduces **acetate** production. Both results may indicate that the flux through Ppc (from PEP to OAA) is reasonably significant under those conditions, and thus, the modulation of Ppc expression level may affect the utilization of PEP. However, in the present invention, deleting the chromosomal ppc gene did not have a positive effect on DAHP production, suggesting that the flux through Ppc is not important in the methods of the present invention.

Detail Description Paragraph - DETX (56):

[0083] One preferred embodiment of the present invention encompasses modification of a host cell to cause overexpression of an enzyme having the catalytic properties of naturally derived Pps, and, thereby maximizing the yield of DAHP to near theoretical yields. Enzymes having the catalytic activity of Pps include, but are not limited to, Pps produced by expression in whole cells of a naturally derived **pps gene,** enzymes produced by expression in whole cells of a naturally derived **pps gene** modified by sequence deletion or addition so that the expressed enzyme has an amino acid **sequence that varies from unmodified Pps,** abzymes produced to have catalytic sites with steric and electronic properties corresponding to catalytic sites of Pps, or other proteins produced to have the capability of catalyzing the conversion of pyruvate to PEP by any other art recognized means.

Detail Description Paragraph - DETX (58):

[0085] Additionally, the transformation of DNA, including the **pps gene,** into

microorganisms engineered for the overexpression of other substrates, and/or overexpression or derepression of enzymes in the pentose phosphate or common aromatic pathway can be used to tailor the microorganism to achieve near theoretical yields of such DAHP metabolites as tyrosine, tryptophan, phenylalanine, and other aromatic metabolites such as indigo, catechol and quinoid organics such as quinic acid, benzoquinone, and hydroquinone.

Detail Description Paragraph - DETX (73):

[0100] Plasmid pPS341 was constructed by cloning a fragment of E. coli chromosomal DNA containing **pps gene** into an IPTG-inducible expression vector pUHE23-2 (a pBR322 derivative) as taught by Patnaik et al., and the contents of which are herein incorporated by reference. Plasmid pPS341X1 containing the inactive **gene product of pps** was constructed by codon insertion mutagenesis, the details of which are fully described in Patnaik et al. The **pps gene** on pPS341 was inserted with a Mu dII1734 lac.sup.+ Km.sup.r (MudK) according to published protocol of Castiho et al., the contents of which are herein incorporated by reference. Briefly, a Mu lysate was made from a donor strain POII1734/pPS341, which was lysogenized by the mini-Mu element and a Mu cts. The lysate was used to infect a Mu lysogen of HG4 pps pck, and colonies were selected for Ap.sup.r and Km.sup.r simultaneously to ensure that the mini-Mu element hopped to the plasmid. Colonies were further screened for Pps.sup.- phenotype (inability to grow on pyruvate). Restriction analysis of plasmid DNA further confirmed the insertion of the MudK element into the **pps gene** on plasmid pPS341. 20% of these selected colonies showed IPTG-dependent expression of .beta.-galactosidase, indicating an in-frame insertion. Plasmid from one such colony was named pPS1734, which was then linearized at the ScaI site, and then transformed into strain JC7623 recB21 recC22 sbcB15. Transformants were selected for Km.sup.r and scored for Ap sensitivity. Such colonies presumably contained pps::MudK on the chromosome. By use of P1 transduction, this locus was moved to AB2847 and Km.sup.r transductants were further screened for inability to grow on pyruvate. One such colony was designated JCL1362 and used for later studies. The MudK insertion into chromosomal pps was further confirmed by cotransduction frequency (89%) with Tet.sup.r marker from strain CAG12151 zdh-925::Tn10.

Detail Description Paragraph - DETX (76):

[0103] The plasmid pPS706 was constructed by inserting a 2.4 kb PCR fragment containing the promoter-less **pps gene** into the vector pJF118EH. The primers were designed from the published **pps sequence** and contained an EcoRI site and a .phi.10 ribosome binding site upstream of the **pps sequence** and a BamHI site downstream of the sequence. The PCR product was then cloned into the EcoRI and BamHI sites of pJF118EH. Positive clones were selected based on complementation of HG4 pps for growth on pyruvate. Expression of pps from this construct is controlled by the tac promoter inducible by IPTG.

Detail Description Paragraph - DETX (77):

[0104] The plasmid pPSL706 was then constructed from pPS706 as shown in FIG. 5. Briefly, a ScaI/EcoRI fragment containing the **pps gene** was cut from pPS706 and purified from the restriction buffer. This fragment was then cloned into a purified ScaI-EcoRI fragment containing the luxI' promoter from pGS103, kindly

given to the inventor by Tom Baldwin. Department of Biochemistry and Biophysics, Texas A&M University. Expression using this system is controlled by the autoinducer (AI) in the culture media. pPSL706 is ampicillin resistant and compatible with other pACYC184 derivatives such as pRW5 and pATI. The strains and plasmids used are summarized in Table I and Table II.

Detail Description Paragraph - DETX (90):

[0117] To gain insight into the metabolic flux distribution, the culture broth was analyzed for fermentation byproducts by use of HPLC. Samples were taken from cultures in glucose media with varying activities of Pps, AroG, and TktA. Results indicate that the host strain AB2847 produced **acetate,** succinate, and formate as the major byproducts when neither AroG nor Pps was overexpressed. Production of these acids generally decreased with the increase in IPTG concentration, except formate. This decrease correlates with the increase in DAHP production. When AB2847/pAT1/pPS706 was cultured in glucose with IPTG concentration beyond 50 mM, the broth had undetectable levels of these acids (data not shown). While levels of formic and acetic acid decreased with increase in Pps activity, succinic acid either remained constant (0 .mu.M IPTG) or increased (10.50 .mu.M IPTG) with an increase in Pps activity. This increase could be contributed to Pps induced increase in PEP level, which is spilled over through PEP carboxylase and eventually to succinate.

Detail Description Paragraph - DETX (93):

[0119] This example demonstrates that the E. Coli AB2847 is unable to utilize DAHP, and accumulates DAHP in the medium if DAHP synthase is overexpressed. This strain was used as a host for detecting the flux committed to the aromatic pathways. Since Draths et al. (Draths, K. M., D. L. Pompliano, D. L. Conley, J. W. Frost, A. Berry, G. L. Disbrow, R. J. Staversky, and J. C. Lievense, "Biocatalytic synthesis of aromatics from D-glucose: The role of transketolase," J. Am. Chem. Soc., 1992, 114, 3956-3962) have shown a possible limitation in the production of DAHP by E4P, pATI (containing both aroG.sup.fbr and tktA) was transformed into AB2847 to eliminate the limitation of E4P. To test whether PEP supply limits DAHP production, PEP synthase (Pps) was overexpressed in AB2847/pAT1 by transforming plasmid pPS341 into this strain. 20-70 copies of the **pps gene** were expressed in the host cells. As a control, pPS341 was substituted by pPS341X1, which encodes an inactive, but stable **pps gene** product. The use of the inactive Pps control allowed discrimination between the effect of Pps activity and that of protein overexpression. AB2847/pAT1/pUHE23-2 and AB2847/pAT1 were also used without any other plasmid as additional controls to identify the effect of the cloning vector, pUHE23-2, on DAHP production.

Detail Description Paragraph - DETX (98):

[0123] As shown above, Pps overexpression improved DAHP production from glucose. We were interested to know whether the basal level of Pps expression in glucose medium contributed to the production of DAHP. Therefore, the chromosomal **pps gene** in strain AB2847 was knocked out. The resulting strain (JCL1362) was used as the host to repeat the above experiments. Results show that inactivation of chromosomal pps did not significantly affect the DAHP production in strains containing pRW5 or pATI (FIG. 2B). Therefore, the basal

level of pps expression in glucose medium did not contribute to the DAHP production.

Detail Description Paragraph - DETX (103):

[0127] To produce tryptophan, strain ATCC31743 which contains chromosomal markers such as trpR .DELTA.(trpAE) tna can be used as a host. This strain also contains a plasmid pSC102trp which harbors trpAE operon. Plasmids pATI and pPS341 (or pPS706 or pPSL706) can be transformed into this strain. The serA gene can be cloned into any of the plasmids. Alternatively, these cloned **genes (trpAE, aroG, tktt, pps** or serA) can be consolidated to one or two plasmids. The resulting strain was grown in MT medium which contains, per liter: KH.sub.2PO.sub.4, 3 g: K.sub.2HPO.sub.4, 3 g; K.sub.2HPO.sub.4, 7 g; NH.sub.4CL, 3g; MgSO.sub.4, 0.2 g; FeSO.sub.4 7H.sub.2O), 10 mg, glucose, 0 to 30 g.

Detail Description Paragraph - DETX (117):

[0137] Quinoid organics can be derived from dehydroquinate which is a down-stream metabolite of DAHP. To produce quinic acid, E. coli AB2848 aroD harboring pTW8090A which contains the gene qad (quinic acid dehydrogenase from Klebsiella pneumoniae) (ref: Draths, Ward, and Frost, 1992, JACS, 114, 9725-9726), and pKD136 (ref: same as above) which contains tkt, aroF, and aroB genes can be used as a host. The **pps gene** can be cloned into one of these plasmids and be simultaneously overexpressed. It has been reported that at least 80 mM of D-glucose can be converted into 25 mM of quinic acid. After cell removal, quinic acid in the supernatant can be converted into benzoquinone after addition of sulfuric acid and technical grade manganese (IV) dioxide and heating at 100.degree. C. for 1 h. In the absence of acidification, aqueous solutions of purified quinic acid were converted to hydroquinone in 10% yield upon heating at 100.degree. C. for 18 h with technical grade manganese dioxide.

Claims Text - CLTX (11):

10. A process for the production of DAHP which comprises cultivating a microorganism in a nutrient medium and overexpressing the **Pps gene**.

Claims Text - CLTX (12):

11. The process of claim 10 wherein the step of overexpressing the **Pps gene** comprises transforming a plasmid selected from the group consisting of pPS341, pPSL706, and pPS706 the microorganism.

Claims Text - CLTX (15):

14. A culture containing a microorganism modified with DNA comprising a **pps gene,** said culture being capable of producing increased levels of DAHP upon fermentation in an aqueous nutrient medium containing assimilable sources of carbon, nitrogen and inorganic substances.

US-PAT-NO: 6630332

DOCUMENT-IDENTIFIER: US 6630332 B2

TITLE: Process for the fermentative preparation of L-threonine

DATE-ISSUED: October 7, 2003

INVENTOR-INFORMATION:

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Molenaar; Douwe	Baesweler	N/A	N/A	DE

APPL-NO: 09/ 801042

DATE FILED: March 8, 2001

PARENT-CASE:

This application claims the benefit of Provisional Application No. 60/229,329, filed Sep. 1, 2000.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	100 34 833	July 18, 2000
DE	101 03 874	January 30, 2001

US-CL-CURRENT: 435/189, 435/183 , 435/252.3 , 435/252.32 , 435/320.1 , 435/69.1 , 530/350 , 536/23.2

ABSTRACT:

The invention provides a process for the fermentative preparation of L-threonine using Enterobacteriaceae which in particular already produce L-threonine and in which the nucleotide sequence(s) which code(s) for the mqo gene are enhanced, in particular over-expressed.

13 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

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Brief Summary Text - BSTX (32):

Thus, for example, one or more genes chosen from the group consisting of the thrABC operon which codes for aspartate kinase, homoserine dehydrogenase, homoserine kinase and threonine synthase (U.S. Pat. No. 4,278,765), the pyc gene which codes for pyruvate carboxylase (DE-A-19 831 609), the **pps gene which codes for phosphoenol pyruvate synthase** (Molecular and General Genetics 231:332 (1992)), the ppc gene which codes for phosphoenol pyruvate carboxylase (Gene 31:279-283 (1984)), the genes pntA and pntB which code for transhydrogenase (European Journal of Biochemistry 158:647-653 (1986)), the rhtB gene which imparts homoserine resistance (EP-A-0994190) the rhtC gene which imparts threonine resistance (EP-A-1013765), and the gdhA gene which codes for glutamate dehydrogenase (Gene 27:193-199 (1984))

Detailed Description Text - DETX (43):

Five times, 200 ml LB medium were treated with 100 .mu.g/ml carbenicillin and 100 .mu.M isopropyl .beta.-D-thiogalactoside (IPTG), inoculated with in each case a colony of the strain MC4100.DELTA.mqo/pUCH2 and in each case cultured in 1 l conical flasks for 16 hours at 37.degree. C. and 200 revolutions per minute. The cells were washed twice in buffer A (50 mM hepes, 10 mM potassium **acetate,** 10 mM CaCl.sub.2, 5 mM MgCl.sub.2, adjusted to pH 7.5 with NaOH) at 4.degree. C. and resuspended in 40 ml of the same buffer. The cells were then broken down twice in a precooled French Pressure Cell from Spectronic Unicam (Rochester, N.Y., USA) under 69 MPa (mega-Pascal). The cell debris was then sedimented twice in a centrifuge at 4.degree. C. for 10 minutes at 10000.times.g. The supernatant was then centrifuged for 30 minutes at 75000.times.g and 4.degree. C. The membrane pellet was resuspended with the same volume of buffer B (50 mM Na phosphate, 200 mM NaCl, pH 7.5) and centrifuged again for 30 minutes at 75000.times.g and 4.degree. C. The pellet was then resuspended with 1 ml buffer B. The histidine-tagged malate:quinone oxidoreductase protein was purified in two steps.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	7	glnap$8	USPAT; US-PGPUB	2003/10/09 15:58
2	L2	347110	acetyl adj phosphate or acetylphosphate or acetate	USPAT; US-PGPUB	2003/10/09 15:59
3	L3	83953	promoter$1	USPAT; US-PGPUB	2003/10/09 15:59
4	L4	1448552	induc$8 or regulat$8 or activat$8 or modulat$8	USPAT; US-PGPUB	2003/10/09 15:59
5	L5	66	2 near10 4 near10 3	USPAT; US-PGPUB	2003/10/09 15:59
6	L6	10013	pps or phosphoenol adj pyruvate adj synthase$1	USPAT; US-PGPUB	2003/10/09 16:02
7	L7	98	6 near4 (gene$1 or sequence$1)	USPAT; US-PGPUB	2003/10/09 16:03
8	L8	24	7 same (overexpress$ or amplif$10 or increas$8)	USPAT; US-PGPUB	2003/10/09 16:04
9	L9	21	7 and 2	USPAT; US-PGPUB	2003/10/09 16:08
10	L10	7012	lycopene$1 or isoprenoid$1 or carotene$1 or astaxanthin$1	USPAT; US-PGPUB	2003/10/09 16:10
11	L11	1	6 same 10	USPAT; US-PGPUB	2003/10/09 16:10
12	L12	4074	10 and 2	USPAT; US-PGPUB	2003/10/09 16:11
13	L13	13	12 and 6	USPAT; US-PGPUB	2003/10/09 16:11

PGPUB-DOCUMENT-NUMBER: 20030119715

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DOCUMENT-IDENTIFIER: US 20030119715 A1

TITLE: Treatment of hyperproliferative diseases

PUBLICATION-DATE: June 26, 2003

INVENTOR-INFORMATION:

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RELATED-US-APPL-DATA:

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parent continuation-in-part-of PCT/GB01/03694 20010817 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	0020351.3	2000GB-0020351.3	August 17, 2000

US-CL-CURRENT: 514/1

ABSTRACT:

We describe methods and compositions for treating a patient suffering from a hyperproliferative disorder or photoageing. Our methods involve blocking the activity of a retinol binding protein receptor (RBPr) in cells of the patient, and/or administering to the patient an antagonist of a retinol binding protein receptor (RBPr) and/or lowering the endogenous level of retinoic acid (RA) in cells of said patient.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of international application PCT/GB01/03694, filed Aug. 17, 2001 and claims priority from Great Britian Application No. 0020351.3, filed Aug. 17, 2000. The above-mentioned applications, as well as all documents cited herein and documents referenced or cited in documents cited herein, are hereby incorporated herein by reference.

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Detail Description Paragraph - DETX (43):

[0063] "Retinoids" are a class of compounds consisting of four **isoprenoid** units joined in a head-to-tail manner. All retinoids may be formally derived from a monocyclic parent compound containing five carbon-carbon double bonds and a functional group at the terminus of the acyclic portion. Furthermore, several synthetic compounds with retinoic acid-like activity have recently been developed and are included in the term `retinoids`, e.g. the arotenoids. As employed herein, the term "retinoids" refers to naturally occurring compounds with vitamin A activity, synthetic analogs, and various metabolites thereof.

Detail Description Paragraph - DETX (44):

[0064] Numerous retinoids have been identified, as described, for example, by Sporn, Roberts and Goodman in the two volume treatise entitled The Retinoids (Academic Press, N.Y., 1984), to which the reader is directed for further detail. Exemplary retinoids include retinol, retinyl **acetate,** retinyl hexadecanoate, .alpha.-retinyl, 4,14-retroretinol, deoxyretinol, anhydroretinol, 3,4-didehydroretinol, 15,15-dimethyl retinol, retinyl methyl ether, retinyl phosphate, mannosyl retinyl phosphate, retinol thioacetate, retinal (retinaldehyde), 3,4-didehydroretinal, retinylidene acetylacetone, retinylidene-1,3-cyclopentanedione, retinal oxime, retinaldehyde acetylhydrazone, retinoic acid, 4-hydroxyretinoic acid, 4-oxoretinoic acid, 5,6-dihydroretinoic acid, 5,6-epoxyretinoic acid, 5,8-epoxyretinoic acid, the open-chain C.sub.20 analog of retinoic acid (i.e., (all-E-3,7,11,15-tetramethyl-2,4,6,8,10,12,14-hexadecaheptaenoic acid), 7,8-didehydroretinoic acid, 7,8-dihydroretinoic acid, "Acid" (E,E)-3-methyl-5-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2,4-pentanedioic acid), "C.sub.17 Acid" ((E,E,E)-5-methyl-7-(2,6,6-trimethyl-1-cyclohexen--1-yl)-2,4,6-hepatrienoic acid), "C.sub.22 Acid" (14'-apo-.gamma., psi.-carotenoic acid), retinoic acid esters (e.g., methyl ester, ethyl ester, etc.), retinoic acid ethylamide, retinoic acid 2-hydroxyethylamide, methyl retinone, "C.sub.18 Ketone" 6-methyl-8-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3,5,7-ocatrien-2-one), and the like.

Detail Description Paragraph - DETX (48):

[0068] The body's requirement for vitamin A (retinol) must be satisfied by an adequate dietary intake. Vitamin A is obtained from two sources, firstly as retinyl esters within animal fats and secondly as .beta.**-carotene** from vegetable sources. .beta.**-carotene** is a member of a family of molecules known as carotenoids. .beta.**-carotene** is also referred to as the provitaminn form of vitamin A.

Detail Description Paragraph - DETX (49):

[0069] Ingested .beta.**-carotene** is cleaved in the lumen of the intestine by .beta.**-carotene** dioxygenase to yield retinal. Retinal is reduced to retinol by retinaldehyde reductase, an NADPH requiring enzyme within the intestines. Retinol is esterified mainly to palmitic acid and delivered to the blood via

chylomicrons. The uptake of chylomicron remnants by the liver results in delivery of retinol to this organ for storage as a lipid ester within lipocytes or stellate cells. Transport of retinol from the liver to extrahepatic tissues occurs by binding of hydrolyzed retinol to retinol binding protein (RBP). The retinol-retinol binding protein complex is then transported to the cell surface within the Golgi and secreted.

Detail Description Paragraph - DETX (121):

[0141] Tissue and species responses to **PPs** depend on pharmacokinetics, relative abundance of PPAR isotypes, nature of PPRE in the upstream regions of target genes, the extent of competition or cross-talk among nuclear transcription factors for PPAR heterodimerization partner retinoid X receptor and the modulating role of coactivators and corepressors on ligand-dependent transcription of PPARs

Detail Description Paragraph - DETX (224):

[0244] Although ageing has been thought to be irreversible, studies made during the last decade have shown that some topical compounds and surgical procedures can improve age-related skin damage (Griffiths et al., 1995, Archives of Dermatology 131, 1037-1044; Roger & Fuleihan, 1995, Face lift and adjunctive procedures in the treatment of photodamaged skin. In Photodamage. Gilchrest B A ed. 1995; Blackwell Science, 259-285.; Pierard et al., 1996, Maturitas 23, 273-277; Pierard et al 1997, Dermatology 194, 398-401). Drug treatment consists of sunscreens, retinoids, antioxidants including vitamin C and E and beta-**carotene**, alpha-hydroxyacids and oestrogen (Humphreys et al., 1996, Journal of American Academy of Dermatology 34, 638-644.; Thibault et al., 1998, Dermatology Surgery 24, 573-577; Weiss et al 1988, JAMA 259, 527-532.). A variety of topical prescription and nonprescription agents are widely available for improving photodamaged skin, the efficacy of which is unclear. Topical retinoic acid treatment results in the increased synthesis of collagens in the dermis and effacement of wrinkles (Griffiths et al., 1993, New England Journal of Medicine 329, 530-535; Kligman, 1987, J Invest Dermatol 88, 12s-17s; Kligman et al., 1984, Connect Tissue Res 12, 139-50). Application of retinoic acid also produces a deposition of linear elastic fibres replacing the tortuous elastic fibres produced by UV irradiation (Tsukahara et al., 1999, Br J Dermatol 140, 1048-1053).

Detail Description Paragraph - DETX (250):

[0270] The pharmaceutical compositions according to our invention include those suitable for topical and oral administration, with topical formulations being preferred where the tissue affected is primarily the skin or epidermis (for example, psoriasis and other epidermal hyperproliferative diseases, photoageing, skin cancer, etc). The topical formulations include those pharmaceutical forms in which the composition is applied externally by direct contact with the skin surface to be treated. A conventional pharmaceutical form for topical application includes a soak, an ointment, a cream, a lotion, a paste, a gel, a stick, a spray, an aerosol, a bath oil, a solution and the like. Topical therapy is delivered by various vehicles, the choice of vehicle can be important and generally is related to whether an acute or chronic disease is to be treated. As an example, an acute skin proliferation disease

generally is treated with aqueous drying preparations, whereas chronic skin proliferation disease is treated with hydrating preparations. Soaks are the easiest method of drying acute moist eruptions. Lotions (powder in water suspension) and solutions (medications dissolved in a solvent) are ideal for hairy and intertriginous areas. Ointments or water-in-oil emulsions, are the most effective hydrating agents, appropriate for dry scaly eruptions, but are greasy and depending upon the site of the lesion sometimes undesirable. As appropriate, they can be applied in combination with a bandage, particularly when it is desirable to increase penetration of the retinol binding protein receptor antagonist or blocking agent or retinoic acid synthesis enzyme (including retinol dehydrogenase and retinal dehydrogenase) inhibitor composition into a lesion. Creams or oil-in-water emulsions and gels are absorbable and are the most cosmetically acceptable to the patient. (Guzzo et al, in Goodman & Gilman's Pharmacological Basis of Therapeutics, 9th Ed., p. 1593-15950 (1996)). Cream formulations generally include components such as petroleum, lanolin, polyethylene glycols, mineral oil, glycerin, isopropyl palmitate, glyceryl stearate, cetearyl alcohol, tocopheryl **acetate,** isopropyl myristate, lanolin alcohol, simethicone, carbomen, methylchlorisothiazolinone, methylisothiazolinone, cyclomethicone and hydroxypropyl methylcellulose, as well as mixtures thereof.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030114353 A1

TITLE: Store operated calcium influx inhibitors and methods of use

PUBLICATION-DATE: June 19, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 160977

DATE FILED: May 31, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60295129 20010531 US

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US-CL-CURRENT: 514/1

ABSTRACT:

The present invention provides store operated calcium influx inhibitor compounds, pharmaceutical compositions, and methods of use. The compounds are useful for treating an inflammatory disease or treating an inflammatory reaction. Preferably, compounds, compositions and methods of this invention are used for treatment of inflammatory skin, pulmonary, musculoskeletal, and gastrointestinal diseases, as well as autoimmune disorders, transplantation treatment, and osteoporosis.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Patent Application Nos. 60/295,129 and 60/295,124, both filed on May 31, 2001, the disclosures of which are hereby incorporated by reference in their entireties for all purposes.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (16):
[0016] If the inflammation occurs at the urogenital epithelium, vaginitis,

interstitial cystitis, and the like, may develop. Vaginitis is a common gynecologic disorder that is responsible for 10 million office visits to physicians each year. Infectious vaginitis is the most common cause of a vaginal discharge, but other important diagnostic considerations include infectious cervicitis, a physiologic discharge, atrophic vaginitis, and allergic or irritant vaginitis (see, Quan M, Clin. Cornerstone, 3(1):36-47, 2000). Interstitial cystitis (IC) is a disorder of unknown etiology with few effective therapies. Interstitial cystitis (IC) is a painful, sterile, disorder of the urinary bladder characterized by urgency, frequency, nocturia and pain. IC occurs primarily in women but also in men with recent findings indicating that chronic, abacterial prostatitis may be a variant of this condition. The prevalence of IC has ranged from about 8-60 cases/100,000 female patients depending on the population evaluated. About 10% of patients have severe symptoms that are associated with Hunner's ulcers on bladder biopsy; the rest could be grouped in those with or without bladder inflammation. Symptoms of IC are exacerbated by stress, certain foods and ovulatory hormones. Many patients also experience allergies, irritable bowel syndrome (IBS) and migraines. There have been various reports indicating dysfunction of the bladder glycosaminoglycan (GAG) protective layer and many publications showing a high number of activated bladder mast cells. Increasing evidence suggests that neurogenic inflammation and/or neuropathic pain is a major component of IC pathophysiology. Approved treatments so far include intravesical administration of dimethylsulphoxide (DMSO) or oral pentosanpolysulphate (**PPS**). However, none of these therapies were proven effective in treating a large proportion of patients afflicted with IC.

Brief Description of Drawings Paragraph - DRTX (15):

[0142] FIG. 14 illustrates the effects of two crystal fractions of 1079-76-3 and 1079-79-4 on NFAT-luciferase activity in Jurkat stable cells stimulated with ionomycin and phorbol-12-myristate, 13**-acetate** in peripheral mononuclear cells (PMA).

Brief Description of Drawings Paragraph - DRTX (16):

[0143] FIG. 15 illustrates the effects of two crystal fractions of 1079-76-3 and 1079-79-4 on NFAT-luciferase activity in Jurkat cells stimulated with thapsigargin and phorbol-12-myristate, 13**-acetate** in peripheral mononuclear cells (PMA).

Detail Description Paragraph - DETX (45):

[0191] The novel mechanism of SOC inhibition 100 of the present invention is set forth in FIG. 1. This illustration is merely an example and is not intended to be limiting. Those of skill in the art will recognize alternatives, modifications and variations. As shown therein, statins in the open-chain form 101 are inhibitors of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase 105. The high-potency delayed effects of the HMG-CoA reductase mechanism 103 is characterized in that HMG-CoA reductase 105 is the rate-limiting step in the biosynthesis of mevalonate 112, the precursor of cholesterol 115 synthesis and a variety of non-sterol **isoprenoid** products such as geranylgeranyl pyrophosphate 107 and farnesyl pyrophosphate 108. Rho proteins require geranylgeranyl pyrophosphate to function. Rho has been

implicated in endothelial nitric oxide synthase (eNOS) suppression, lymphocyte function-associated antigen-1 (LFA-1) activation, major histocompatibility complex class II (MHC-II) induction, cytokine production, cell cycle progression, cell proliferation. Statins have been shown to reduce the risk of cardiovascular mortality and morbidity, and have become the most commonly used drugs for the treatment of hypercholesterolemia and arteriosclerosis. In contrast, the SOC inhibitor mechanism 100 of the present invention acts by blocking calcium influx 122, which in turn, blocks calcium dependent T cell activation 125 and mast cell degranulation 126. Histamine release 121 is also inhibited. As this pathway 100 depends on higher concentrations of active 127 (low potency) compared to the HMG-CoA reductase mechanism 101, the therapeutic utility of the SOC inhibitors is preferably achieved using high local concentrations for immediate effect 127. The SOC inhibitors of the present invention inhibit calcium influx into cells, thereby inhibiting calcium dependent activation.

Detail Description Paragraph - DETX (53):

[0199] Statins potently inhibit the enzyme HMG-CoA reductase that catalyzes the conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonate, a key intermediate in the biosynthesis of cholesterol and other non-sterol **isoprenoid** products. HMG-CoA reductase is specifically inhibited by the open .beta.-hydroxy form of lovastatin. The anti-proliferative and anti-inflammatory effects of HMG-CoA reductase inhibitors are mediated by suppression of the **isoprenoids** famesyl pyrophosphate (F-PP) and geranylgeranyl pyrophosphate (GG-PP), used in the post-translational modification of signaling proteins, e.g., small GTPases of the Ras and Rho families. Rho geranylgeranylation plays roles in T cell proliferation, eNOS regulation, LFA-1 activation, the oxidative burst, chemotaxis, cytokine synthesis, and IFN.gamma.-induced MHC class II expression. The effects of HMG-CoA reductase inhibitors on these processes are somewhat delayed since depletion of the **isoprenoid** pools occurs over a period of hours.

Detail Description Paragraph - DETX (65):

[0211] The nuclear factor of activated T cells (NFAT) proteins are a family of transcription factors whose activation is controlled by calcineurin, a calcium-dependent protein phosphatase (Rao A et al., Annu. Rev. Immunol. 15:707-47, 1997; Stankunas K et al., Cold Spring Harb. Symp. Quant. Biol. 64:505-16, 1999). Originally identified in T cells as inducers of cytokine gene expression, NFAT proteins play varied roles in cells outside of the immune system (Horsley V and Pavlath G K, J. Cell Biol. 156:771-4, 2002; Graef I A et al., Curr. Opin. Genet. Dev. 11:505-12, 2001). Recently, using immunofluorescence/confocal microscopy, cyclosporin A and tacrolimus were shown to block the nuclear translocation of calcineurin and NFAT in cultured keratinocytes (A1-Daraji W I et al., J. Invest. Dermatol. 118:779-88, 2002). The results showed that a variety of cell types in normal and psoriatic skin expressed calcineurin and NFAT1, but expression was particularly prominent in keratinocytes. The principal cyclosporin A and tacrolimus binding proteins cyclophilin A and FKBP12 were also expressed in keratinocytes and nonimmune cells in skin. NFAT1 was predominantly nuclear in normal basal epidermal keratinocytes. Increased nuclear localization of NFAT1 was observed in suprabasal keratinocytes within lesional and to a lesser extent nonlesional

psoriatic epidermis compared to normal skin, suggesting increased activation of calcineurin in psoriatic epidermal keratinocytes. Agonists that induce keratinocyte differentiation, specifically 12-0-tetradecanoyl-phorbol-13-**acetate** (TPA) plus ionomycin, raised intracellular calcium, induced nuclear translocation of NFAT1 and calcineurin in keratinocytes, and was inhibited by pretreatment with cyclosporin A or tacrolimus. In contrast, in human dermal fibroblasts, TPA plus ionomycin or TPA did not significantly alter the proportion of nuclear-associated NFAT1. These results indicate that calcineurin is functionally active in human keratinocytes by inducing nuclear translocation of NFAT1, and that regulation of NFAT1 nuclear translocation in skin is cell type specific. Inhibition of this pathway in epidermal keratinocytes may account, in part, for the therapeutic effect of cyclosporin A and tacrolimus in skin diseases such as psoriasis. SOC inhibitors which can effectively inhibit NFAT activation provide an alternative pharmacological treatment for inflammatory conditions such as psoriasis.

Detail Description Paragraph - DETX (135):

[0278] The effects of the novel compounds 1079-76-3C and 1079-76-4C on nuclear factor of activated T-cell (NFAT) activation were investigated in Jurkat cells (human leukemic T-cells). The novel compounds inhibited the expression of an NFAT-regulated reporter gene stably transfected into Jurkat cells, stimulated with ionomycin (I) and phorbol-12-myristate, 13-**acetate** (PMA) (see, FIG. 13), or with thapsigargin (TG)+PMA (see, FIG. 14).

Detail Description Paragraph - DETX (177):

[0317] For these compositions, the solubility of a layer of the coating material in gastric fluid and intestinal fluid determines the where the coating will dissolve, and thus determines the region of the gastrointestinal tract in which the drug will be absorbed. Cellulose phthalate, cellulose **acetate** phthalate, polyacrylates or methacrylates can be used as coating materials to produce tablets and granules resistant to gastric fluid and soluble in intestinal fluid.

Detail Description Paragraph - DETX (182):

[0322] The compound prepared and isolated in Example 1 is placed in a large-volume mortar, and a 10% cellulose **acetate** phthalate--acetone solution is added drop by drop and kneaded until the acetone is nearly evaporated. A 10% (w/v) acacia solution is slowly added, and the mixture is kneaded for 7 minutes. The mass is granulated in a wet granulator and dried in a fluid-bed drier at 50.degree. C. for 30 minutes. The granules are sieved, and granule sizes between 500 .mu.m and 177 .mu.m are used for tabletting. One to one mixtures of granules and microcrystalline cellulose are tabletted in a single-punch machine to produce tablets composed of cellulose **acetate** phthalate-coated granules of the compound from Example 1.

Detail Description Paragraph - DETX (189):

[0329] Lovastatin is placed in a large-volume mortar, and a 10% cellulose **acetate** phthalate--acetone solution is added drop by drop and kneaded until the

acetone is nearly evaporated. A 10% (w/v) acacia solution is slowly added, and the mixture is kneaded for 7 minutes. The mass is granulated in a wet granulator and dried in a fluid-bed drier at 50.degree. C. for 30 minutes. The granules are sieved, and granule sizes between 500 .mu.m and 177 .mu.m are used for tabletting. One to one mixtures of granules and microcrystalline cellulose are tabletted in a single-punch machine to produce tablets cores composed of enteric-coated lovastatin granules.

US-PAT-NO: 6627636

DOCUMENT-IDENTIFIER: US 6627636 B2

TITLE: HMG-CoA reductase inhibitors and method

DATE-ISSUED: September 30, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Robl; Jeffrey A.	Newtown	PA	N/A	N/A

APPL-NO: 10/ 007407

DATE FILED: December 4, 2001

PARENT-CASE:

This application is a continuation-in-part of U.S. application Ser. No. 09/875,155 filed Jun. 6, 2001, abandoned which application claims priority from U.S. provisional application No. 60/211,595, filed Jun. 15, 2000.

US-CL-CURRENT: 514/291, 514/213.01 , 514/292 , 540/577 , 546/80 , 546/81 , 546/89 , 546/93

ABSTRACT:

Compounds of the following structure are HMG CoA reductase inhibitors and thus are active in inhibiting cholesterol biosynthesis, modulating blood serum lipids such as lowering LDL cholesterol and/or increasing HDl cholesterol, and treating hyperlipidemia, hypercholesterolemia, hypertriglyceridemia and atherosclerosis ##STR1##

and pharmaceutically acceptable salts thereof, wherein X is O, S, SO, SO.sub.2 or NR.sub.7 ; Z is ##STR2##

n is 0 or 1; R.sub.1 and R.sub.2 are the same or different and are independently selected from alkyl, arylalkyl, cycloalkyl, alkenyl, cycloalkenyl, aryl, heteroaryl or cycloheteroalkyl; and R.sub.3 to R.sub.10 are as defined herein.

25 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (115):

The squalene synthetase inhibitors suitable for use herein include, but are not limited to, .alpha.-phosphono-sulfonates disclosed in U.S. Pat. No. 5,712,396, those disclosed by Biller et al, J. Med. Chem., 1988, Vol. 31, No. 10, pp 1869-1871, including **isoprenoid** (phosphinylmethyl)phosphonates as well as other known squalene synthetase inhibitors, for example, as disclosed in U.S. Pat. No. 4,871,721 and 4,924,024 and in Biller, S. A., Neuenschwander, K., Ponpipom, M. M., and Poulter, C. D., Current Pharmaceutical Design, 2, 1-40 (1996).

Brief Summary Text - BSTX (130):

an anti-oxidant such as beta-**carotene,** ascorbic acid, .alpha.-tocopherol or retinol as disclosed in WO 94/15592 as well as Vitamin C and an antihomocysteine agent such as folic acid, a folate, Vitamin B6, Vitamin B12 and Vitamin E;

Brief Summary Text - BSTX (210):

a chondroprotective compound such as a polysulfated glycosaminoglycan (PSGAG), glucosamine, chondroitin sulfate (CS), hyaluronic acid (HA), pentosan polysulfate (**PPS**), doxycycline or minocycline, such as disclosed in EP 970694;

Detailed Description Text - DETX (8):

A mixture of crude Part B compound (17.0 g, 27.3 mmol), ammonium **acetate** (9.34 g, 120 mmol) and copper **acetate** monohydrate (20.54 g, 101 mmol) in glacial acetic acid (100 ml) was refluxed under argon for 19 hours. The mixture was poured into an ice-cold solution of concentrated ammonium hydroxide (85 ml) in water (170 ml) and the bright blue solution was extracted with ether (3.times.200 ml). The combined organic extracts were washed with water (2.times.80 ml) and brine (80 ml), dried (anhydrous Na.sub.2 SO.sub.4), filtered, evaporated to dryness, and dried in vacuo. The crude product (14 g, brown syrup) was chromatographed in two batches, each on a silica gel column (EM, 21/4".times.10") to give the desired product as an off-white solid (4.161 g). An additional 931 mg of product was obtained from chromatography of mixed fractions. Yield: 5.092 g, 46% from compound A). Rf 0.53 (Silica gel; EtOAc:Hexane-1:4; UV) ##STR40##

Detailed Description Text - DETX (9):

A solution of Part C compound (2.515 g, 6.23 mmol) in dry THF (30 ml) was cooled to 0.degree. C. (ice-water bath), treated dropwise with lithium aluminum hydride (1.0 M in THF; 12.5 ml, 12.5 mmol), stirred at 0.degree. C. for 30 minutes then at room temperature for 3 hours. The reaction mixture was cooled to 0.degree. C., treated successively with water (0.5 ml), 15% NaOH (0.5 ml) and water (1.5 ml), stirred at room temperature for 5 minutes then diluted with ethyl **acetate** (50 ml). The slurry was filtered through a Celite.RTM. pad, washing the pad well with ethyl **acetate** (3.times.25 ml). The clear filtrate was evaporated to dryness and dried in vacuo to give the title product. Yield: 2.386 g, white foam (100%). Rf 0.15 (Silica gel; EtOAc:Hexane-1:4; UV) ##STR41##

Detailed Description Text - DETX (10):

A solution of Part D compound (2.27 g, 6.23 mmol) in dry dichloromethane (45 ml) was cooled to 0.degree. C. (ice-water bath) and treated dropwise with phosphorus tribromide (1.0 M in dichloromethane; 12.5 ml, 12.5 mmol). The ice bath was removed and the reaction mixture was stirred at room temperature for 30 minutes after which it was re-cooled to 0.degree. C. and treated dropwise with saturated sodium bicarbonate (70 ml). The mixture was then warmed to room temperature and extracted with ethyl **acetate** (2.times.100 ml). The combined organic extracts were washed with water (2.times.50 ml) and brine (50 ml), re-extracting each aqueous wash with dichloromethane (100 ml). The organic extracts were dried (anhydrous sodium sulfate), filtered, evaporated to dryness and dried in vacuo to give the title product as a white solid. Yield: 2.503 g, (94%). m.p.=169-171.degree. C. Rf 0.58 (Silica gel; EtOAc:Hexane-1:4; UV). ##STR42##

Detailed Description Text - DETX (11):

A solution of diethyl phosphite (0.88 ml, 6.83 mmoles) in dry THF (10 ml) was cooled to -10.degree. C. (acetonitrile-dry ice bath), treated with sodium (bistrimethylsilyl)amide (1.0 M in THF; 6.7 ml, 6.7 mmol) and stirred at -10.degree. C. for 30 minutes. The cooled solution was treated with a solution of Part E compound (2.41 g, 5.68 mmol) in dry THF (20 ml), stirred at -10.degree. C. for 1.0 hour then quenched at -10.degree. C. with water (14 ml). The solution was extracted with ethyl **acetate** (2.times.75 ml) and the combined organic extracts washed with 1.0 M hydrochloric acid (8.0 ml) and brine (10 ml), dried (anhydrous sodium sulfate), filtered, evaporated to dryness and dried in vacuo. The crude product (3.12 g, syrup) was chromatographed on a silica gel column (EM, 5.5 cm.times.12.5 cm) to give the title compound as a syrup. Yield: 2.34 g (85.5%). Rf 0.33 (Silica gel; EtOAc-Hexane-1:1; UV). ##STR43##

Detailed Description Text - DETX (12):

A solution of Part F compound (2.29 g, 4.756 mmol) in dry THF (20 ml) was cooled to -78.degree. C., treated with 2.37 M n-butyllitium (2.41 ml, 5.71 mmol) and stirred at -78.degree. C. for 40 minutes. The solution was treated dropwise via cannula with a -78.degree. C. solution of Part A(I) compound (2.36 g, 9.15 mmol) in dry THF (10 ml), keeping both solutions at -78.degree. C. at all times. The reaction mixture was stirred at -78.degree. C. for 1.0 hr, -10.degree. C. for 1.0 hr and at room temperature for 5 hr, quenched with 25% ammonium chloride solution (12 ml) then extracted with ethyl **acetate** (2.times.100 ml). The combined organic extracts were washed with 25% ammonium chloride solution (12 ml) and brine (12 ml), dried (anhydrous sodium sulfate), filtered, evaporated to dryness and dried in vacuo. The crude product yellow syrup was chromatographed on a silica gel column (EM, 21/4".times.10") to afford the title compound as a syrup. Yield: 878 mg (32%). Rf 0.37 (Silica gel; EtOAc:Hexane-1:4; UV). ##STR44##

Detailed Description Text - DETX (13):

A solution of Part G compound (850 mg, 1.45 mmol) in dry dichloromethane (20 ml) was cooled to 0.degree. C., treated with trifluoroacetic acid (1.85 ml, 24 mmol), stirred at 0.degree. C. for 5 minutes, then at room temperature for 4.5

hours. The reaction mixture was poured slowly into a 1 L flask containing ethyl **acetate** (300 ml) and saturated sodium bicarbonate (40 ml), rinsing the flask with ethyl **acetate** (50 ml). The mixture was stirred well and the phases separated, washing the organic phase with saturated sodium bicarbonate (25 ml) and brine (25 ml). The organic phase was dried over anhydrous sodium sulfate, filtered, evaporated to dryness and dried in vacuo. The crude product mixture was chromatographed on a silica gel column (EM, 1.5".times.12") to give the desired compound as a syrup. Yield: 570 mg (83%). Rf 0.23 (Silica gel; EtOAc:Hexane-1:1; UV) ##STR45##

Detailed Description Text - DETX (110):

(prepared as described in Example 2 Parts A, B and C except methyl isobutyryl **acetate** is substituted for ethyl isobutyryl **acetate**) and toluene (170 mL). The mixture is stirred at 20-25.degree. C. until a clear solution is obtained. A solutin of 65% Red-Al in toluene (57.8 mL, 192.6 mmol) is added and the reaction mixture is heated to 80.degree. C. until complete as determined by HPLC. The reaction mixture is cooled to 20.degree. C. and quenched by pouring it into cold (0-5.degree. C.) 20% HCl (495 mL). Phases are separated and the spent toluene phase is discarded. The pH of the aqueous phase is adjusted from <0 to 4-5 with 10N NaOH. Ethyl **acetate** (500 mL) is added and the pH adjustment continued to 7-8. The phases are separated. The aqueous phase is extracted with additional ethyl **acetate** (2.times.500 mL). The combined rich ethyl **acetate** solution is washed with water (3.times.250 mL) and concentrated under reduced pressure to .about.465 mL. This solution is carried through to the next oxidation step.

Detailed Description Text - DETX (111):

The rich ethyl **acetate** solution is charged from above into a three neck 1-L flask equipped with mechanical stirring, temperature controller, and addition funnel and cooled to 0-5.degree. C. To the slurry, potassium bromide (1.53 g, 12.8 mmol) and TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) (0.20 g, 1.28 mmol) are added. The pH of NaOCl (sodium hypochlorite) solution (212.1 mL) is adjusted to .about.9.1 and added to the slurry at a rate such that the temperature remained at 0-5.degree. C. Stirring is continued at 0-5.degree. C. until the reaction is complete as determined by HPLC. The aqueous phase is extracted with EtOAc (2.times.200 mL). The combined rich organic phase is washed with a 1:1 solution of sat. aq. Na.sub.2 S.sub.2 O.sub.3 (sodium thiosulfate) (75 mL) and water (75 mL) followed by wash of the rich organic phase with 1N NaOH (250 mL). The rich organic phase is washed with water (250 mL) and concentrated to .about.100 mL under reduced pressure. Isopropanol (IPA) (400 mL) is added and the resulting mixture is heated to reflux (80-85.degree. C.). The solution is distilled to a volume of .about.250 mL. Water (50 mL) is added and the crystal slurry is stirred at 70-80.degree. C. for 1 h then allowed to cool to 20-25.degree. C. over at least 1 h. The slurry is held at 20-25.degree. C. for at least 1 h before collecting the solid by filtration on a Buchner funnel. The cake is washed with cold (0.degree. C.) IPA/water (4:1) (2.times.50 mL) and dried to a constant weight under vacuum at 40.degree. C. to afford title aldehyde.

Detailed Description Text - DETX (125):

An N.sub.2 purged 250 mL 3-neck rb flask is charged with Example 27 pyridine derivative (18) (5 g, 13.9 mmol), Example 28 sulfone (16) (6.9 g, 15.3 mmol) and THF (75 mL). The stirred solution is cooled to -74 to -78.degree. C. Slowly a 1M solution of LiHMDS (lithium bis(trimethylsilyl)amide) (15.3 mL, 15.3 mmol) in THF is charged at a rate such that the temperture remained between -70 and -78.degree. C. After addition of the base is complete, the reaciton mixture is warmed to .about.-45.degree. C. over .about.15 minutes. The stirred reaction is quenched at -70.degree. C. by slow addition of sat. aq. NH.sub.4 Cl (7.5 mL) solution and water (38 mL). The dry ice bath is removed and the solution is warmed to 20-25.degree. C. from the reaction mixture. Ethyl **acetate** (50 mL) is added, the mixture agitated, and layers separated. The organic layer is washed with saturated sodium bicarbonate solution (2.times.38 mL) followed by brine (25 mL) and concentrated to a volume of 50 mL. Acetonitrile (50 mL) is added and the solution is concentrated to a volume of 50 mL. This step is repeated. Water (.about.5-6 mL) is slowly added to the hot solution (60-70.degree. C.) until the cloud point is reached. The thin slurry is held for 30 min at high temperature and then slowly cooled over several hours with stirring. The product is filtered, cake is washed with a 5:1 mixture of acetonitrile and water, and dried to afford the title compound.

US-PAT-NO: 6620821

DOCUMENT-IDENTIFIER: US 6620821 B2

TITLE: HMG-CoA reductase inhibitors and method

DATE-ISSUED: September 16, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Robl; Jeffrey A.	Newtown	PA	N/A	N/A

APPL-NO: 10/ 008154

DATE FILED: December 4, 2001

PARENT-CASE:

This application is a continuation-in-part of U.S. application Ser. No. 09/875,218 filed Jun. 6, 2001, abandoned which application claims priority from U.S. provisional application No. 60/211,594, filed Jun. 15, 2000.

US-CL-CURRENT: 514/290, 546/101 , 546/111 , 546/93

ABSTRACT:

Compounds of the following structure are HMG CoA reductase inhibitors and thus are active in inhibiting cholesterol biosynthesis, modulating blood serum lipids, for example, lowering LDL cholesterol and/or increasing HDL cholesterol, and treating hyperlipidemia, dyslipidemia, hormone replacement therapy, hypercholesterolemia, hypertriglyceridemia and atherosclerosis as well as Alzheimer's disease and osteoporosis ##STR1##

and pharmaceutically acceptable salts thereof, ##STR2## n is 0 or 1; x is 0, 1, 2, 3 or 4; y is 0, 1, 2, 3 or 4, provided that at least one of x and y is other than 0; and optionally one or more carbons of (CH.sub.2).sub.x and/or (CH.sub.2).sub.y together with additional carbons form a 3 to 7 membered spirocyclic ring; R.sub.1 and R.sub.2 are the same or different and are independently selected from alkyl, arylalkyl, cycloalkyl, alkenyl, cycloalkenyl, aryl, heteroaryl or cycloheteroalkyl; R.sub.3 is H or lower alkyl; R.sub.4 and R.sub.7 are as defined herein.

18 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (95):

Referring to Scheme 5, the arginine salt of the compounds of formula I of the invention may be prepared by treating alkali metal salt (preferably sodium) Ib with acid (TFA, HCl) to form the acid Ib.sup.6 which is treated with arginine in the presence of suitable solvents such as ethyl alcohol and H.sub.2 O, ethyl **acetate,** acetonitrile and the like, to form arginine salt Ib.sup.7. ##STR33##

Brief Summary Text - BSTX (116):

The squalene synthetase inhibitors suitable for use herein include, but are not limited to, .alpha.-phosphono-sulfonates disclosed in U.S. Pat. No. 5,712,396, those disclosed by Biller et al, J. Med. Chem., 1988, Vol. 31, No. 10, pp 1869-1871, including **isoprenoid** (phosphinyl-methyl)phosphonates as well as other known squalene synthetase inhibitors, for example, as disclosed in U.S. Pat. Nos. 4,871,721 and 4,924,024 and in Biller, S. A., Neuenschwander, K., Ponpipom, M. M., and Poulter, C. D., Current Pharmaceutical Design, 2, 1-40 (1996).

Brief Summary Text - BSTX (125):

The other lipid agent also includes a phytoestrogen compound such as disclosed in WO 00/30665 including isolated soy bean protein, soy protein concentrate or soy flour as well as an isoflavone such as genistein, daidzein, glycitein or equol, or phytosterols, phytostanol or tocotrienol as disclosed in WO 2000/015201; a beta-lactam cholesterol absorption inhibitor such as disclosed in EP 675714; an HDL upregulator such as an LXR agonist, a PPAR .alpha.-agonist and/or an FXR agonist; an .alpha.-glucosidase inhibitor, an aldose reductase inhibitor and/or an LDL catabolism promoter such as disclosed in EP 1022272; a sodium-proton exchange inhibitor such as disclosed in DE 19622222; an LDL-receptor inducer or a steroidal glycoside such as disclosed in U.S. Pat. No. 5,698,527 and GB 2304106; an anti-oxidant such as beta**-carotene,** ascorbic acid, .alpha.-tocopherol or retinol as disclosed in WO 94/15592 as well as Vitamin C and an antihomocysteine agent such as folic acid, a folate, Vitamin B6, Vitamin B12 and Vitamin E; isoniazid as disclosed in WO 97/35576; a cholesterol absorption inhibitor, an HMG-CoA synthase inhibitor, or a lanosterol demethylase inhibitor as disclosed in WO 97/48701; a PPAR .delta. agonist for treating dyslipidemia; or a sterol regulating element binding protein-I (SREBP-1) as disclosed in WO 2000/050574, for example, a sphingolipid, such as ceramide, or neutral sphingomyelenase (N-SMase) or fragment thereof.

Brief Summary Text - BSTX (196):

The HMG CoA reductase compound of the invention may also be employed in combination with a tyrosine kinase inhibitor such as disclosed in WO 2000/053605; the selective androgen receptor modulator suitable for use herein may be LGD-2226 (Ligand); the antiarrhythmic agents suitable for use herein include .beta.-blockers as set out herein including sotalol and amioderome, calcium channel blockers as set out herein including verapamil, nifedipine, amlodipine-besylate, and diltiazem, which may also be used in combination with a debrillator device such as a pace maker; coenzyme Q sub. 10 such as disclosed in U.S. Pat. Nos. 5,316,765, 4,933,165, 4,929,437; an agent that

upregulates type III endothelial cell nitric acid syntase such as disclosed in WO 2000/003746; a chondroprotective compound such as a polysulfated glycosaminoglycan (PSGAG), glucosamine, chondroitin sulfate (CS), hyaluronic acid (HA), pentosan polysulfate (**PPS**), doxycycline or minocycline, such as disclosed in EP 970694; a cyclooxygenase (COX)-2 inhibitor, such as celecoxib (Celebrex.RTM. (Searle)) or rofecoxib (Vioxx.RTM. (Merck)) or a glycoprotein IIa/IIIb receptor antagonist such as disclosed in WO 99/45913 and tirofiban or abciximab; a 5-HT reuptake inhibitor such as disclosed in WO 99/44609; anti-anginal agents such as vasodilators, for example, isosorbide dinitrate, or nitroglycerin; a growth hormone secretagogue such as disclosed in U.S. applications Ser. No. 09/662,448, filed Sep. 14, 2000, and U.S. Provisional application No. 60/203,335, filed May 11, 2000, and MK-677 (Merck), Pfizer's CP-424391 and Lilly's LY 444,711; anti-atherosclerosis agents such as ACAT inhibitors and lipoxygenase inhibitors as described herein and phospholipase A-2 inhibitors such as S-3013 and SB-435,495 (which are also anti-inflammatory agents); anti-infective agents such as quinolones, for example, ciprofloxacin, ofloxacin, and Tequin.RTM. (Bristol-Myers Squibb), macrolides such as erythromycin and clarithromycin (Biaxin.RTM. (Abbott)), and azithromycin (Zithromax (Pfizer)); or an immunosuppressant (for use in transplantations) such as cyclosporine, mycophenolate mofetil, azathioprine and the like.

Detailed Description Text - DETX (5):

To a stirred solution of crude Part C compound (26.9 mmol) in HOAc (128 mL) was added ammonium **acetate** (9.14 g, 118.6 mmol) and copper (II) **acetate** monohydrate (19.7 g, 99.7 mmol). The reaction mixture was heated at reflux under argon overnight. After cooling to room temperature, the reaction mixture was poured into a mixture of NH.sub.4 OH (150 mL) and ice (.sup..about. 300 g), then extracted with Et.sub.2 O (3.times.100 mL). The combined Et.sub.2 O extracts were washed with H.sub.2 O and brine, then dried (Na.sub.2 SO.sub.4), filtered and concentrated in vacuo. Purification by flash chromatography (2:20:80-EtOAc/CH.sub.2 Cl.sub.2 /hexane) gave the title compound as a white foam, 7.7 g, 71% yield (from Part B compound). ##STR45##

Detailed Description Text - DETX (16):

A 1 L 3-necked round bottom flask was flame-dried and then fitted with a mechanical stirrer, an argon-filled balloon, vacuum take-off and a thermocouple. To a stirred slurry of Part (1) compound (7.00 g, 12.9 mmol) in THF (200 mL) at 0.degree. C. was added n-butyllithium solution (5.4 mL, 2.5 M in hexanes, 13.5 mmol) over 20 min. A deep red-orange solution formed. After 30 min, a solution of zinc chloride-N,N,N',N'-tetramethylethylene-diamine complex (dried in vacuo at 60.degree. C. for 2 h, 2.42 g, 13.5 mmol) in THF (100 mL) was added via cannula and stirred 30 min. After 30 min, the resulting solution was cannulated into a solution of Example 2 Part G aldehyde (4.30 g, 16.6 mmol) in THF (20 mL) at room temperature over 20 min. A light orange solution soon formed, followed by a precipitate. After 3 h, the reaction was quenched with brine (50 mL) and water (50 mL) and extracted three times with ethyl **acetate** (100 mL). The organic extracts were combined, dried (MgSO.sub.4) and evaporated. LCMS of the crude material indicated unreacted Part (1) compound and an 89/11 mixture of the desired (E) isomer/undesired (Z) isomer.

Detailed Description Text - DETX (20):

To a solution of 4-fluoro-benzaldehyde (5 g, 40.3 mmol) and ethyl isobutyryl **acetate** (6.5 mL, 40.3 mmol) in benzene (50 mL) was added piperidine (400 .mu.l, 4.04 mmol), followed by acetic acid (100 .mu.l, 1.66 mmol). The reaction was refluxed for 16 hours and partitioned between aqueous HCl (1N, 20 mL) and ethyl **acetate** (50 mL.times.2). The combined organic layers were washed with saturated NaHCO.sub.3 (20 mL), brine (10 ml), and dried over sodium sulfate (Na.sub.2 SO.sub.4). The solvent was removed in vacuo. Distillation at 140.degree. C. at 300 mm Hg afford 9.06 g (85% yield) of compound A as a yellow oil. ESI-LC/MS (M+H).sup.+ =264. ##STR54##

Detailed Description Text - DETX (22):

To a solution of the crude compound B (12 g) in aqueous HOAc (100 mL) was added ammonium **acetate** (9.5 g, 123.2 mmol), followed by cupric **acetate** monohydrate (20.7 g, 113.9 mmol). The reaction was reflux for 20 hours, cooled to room temperature, then poured into a solution of ammonium hydroxide in ice (1 to 1; v/v). The aqueous layer was extracted with ethyl ether (200 mL.times.3). The combined organic layers were washed with water (50 mL) and brine (50 mL). Flash chromatography (10% ethyl **acetate** in hexane) afforded 10.2 g (88% yield) of compound C as white powder. ESI-LC/MS (M+H).sup.+ =404; m.p. 138-140.degree. C. ##STR56##

Detailed Description Text - DETX (23):

To a solution of compound C (10 g, 24.78 mmol) in anhydrous THF (240 mL) at 0.degree. C. was added 1.0 M lithium aluminum hydride in THF (74 mL, 74 mmol). The reaction was stirred at 0.degree. C. for 1 hour and then warmed to room temperature and stirred for 16 hours. The reaction was then cooled to 0.degree. C. and quenched slowly with ice, then sodium hydroxide (10% NaOH, 20 mL). The mixture was extracted with diethyl ether (50 mL.times.2) and filtered. The filter cake was then washed with more ethyl ether (10 mL) and ethyl **acetate** (10 mL). The combined organic layers were washed with water (10 mL) and brine (10 mL), then dried over sodium sulfate (Na.sub.2 SO.sub.4). Flash chromatography (20% ethyl **acetate** in hexane) afforded 6.5 g (73% yield) of compound D. ESI-LC/MS (M+H).sup.+ =362; m.p. 170-171.degree. C. ##STR57##

Detailed Description Text - DETX (24):

To a solution of compound D (8.5 g, 23.54 mmol) in anhydrous CH.sub.2 Cl.sub.2 (100 mL) at 0.degree. C. was slowly added 1.0 M phosphorous tribromide in CH.sub.2 Cl.sub.2 (47 mL, 47.1 mmol) while maintaining the temperature below 10.degree. C. After the addition was complete, the reaction was stirred at 0.degree. C. for 1 hour and then poured into saturated cold NaHCO.sub.3 solution (200 mL) with stirring. The aqueous layer was extracted with CH.sub.2 Cl.sub.2 (50 ml.times.2) and the combined organic layer was washed with water (10 mL) and brine (10 mL), then dried over sodium sulfate (Na.sub.2 SO.sub.4). Flash chromatography (10% ethyl **acetate** in hexane) gave 8.6 g (86% yield) of compound E as a white solid. ESI-LC/MS (M+H).sup.+ =424; m.p. 157-159.degree. C. ##STR58##

Detailed Description Text - DETX (25):

To a solution of diethyl phosphite (785 .mu.L, 6.09 mmol) in anhydrous THF (25 mL) at -10.degree. C. under argon was added a 1.0 M THF solution of sodium hexamethyldisazide (6 mL, 6.09 mmol). The reaction mixture was stirred at -10.degree. C. for 30 minutes. A solution of compound E (2.15 g, 5.08 mmol) in THF was added to the reaction while maintaining the temperature at -10.degree. C. After the addition was complete, the reaction was stirred for 1 hour and quenched with water (20 mL). The aqueous layer was extracted with ethyl **acetate** (30 mL.times.2) and the combined organic layers were washed with iN HCl solution (5 mL). The organic solvent was removed in vacuo. Flash chromatography using 20% to 30% ethyl **acetate** in hexane as eluting afforded 2.29 g (94% yield) of compound F as a white solid. ESI-LC/MS (M+H).sup.+ =482; m.p. 102-105.degree. C. ##STR59##

Detailed Description Text - DETX (28):

To a cooled (-78.degree. C.) solution of compound F (2.02 g, 4.19 mmol) in anhydrous THF (30 mL) under argon was slowly added a 2.5 M THF solution of n-butyllithium (2.1 mL) over a period of 40 minutes. The temperature was maintained below -75.degree. C. during the addition. After the addition was complete, the reaction mixture was stirred for another 40 minutes at -78.degree. C. A solution of compound G (2.2 g, 8.52 mmol) in THF under argon was cannulated into the phosphonate mixture at -78.degree. C. After the addition was complete, the reaction was stirred for 1 hour at -78.degree. C. The reaction was then warmed to -10.degree. C. and stirred for 1 hour and then stirred at room temperature for an additional hour. The mixture was quenched with saturated ammonium chloride solution (5 mL) and extracted with ethyl **acetate** (60 mL.times.3). The combined organic layers were washed with water (10 mL) and brine (10 mL), then dried over sodium sulfate (Na.sub.2 SO.sub.4) and filtered. The filtrate was concentrated in vacuo. Flash chromatography using 5% to 10% ethyl **acetate** in hexane as eluent afforded 1.48 g (60% yield) of compound H as a white solid. ESI-LC/MS (M+H).sup.+ =586; m.p. 148-149.degree. C. ##STR61##

Detailed Description Text - DETX (29):

To a cooled (0.degree. C.) solution of compound H (500 mg, 0.854 mmol) in anhydrous CH.sub.2 Cl.sub.2 (12 mL) under argon was slowly added trifluroacetic acid (987 mL, 12.82 mmol). After the addition was complete, the reaction mixture was allowed to stirred at 0.degree. C. for 10 minutes and at room temperature for 3 hours and then the solvent was removed in vacuo. The reaction mixture was quenched with phosphate solution (pH 7.5, 12 mL) and extracted with CH.sub.2 Cl.sub.2 (20 mL.times.2). The combined organic layers were washed with brine (10 mL), dried over sodium sulfate (Na.sub.2 SO.sub.4), and filtered. The solvent was removed in vacuo. Flash silica gel chromatography using 30% to 50% ethyl **acetate** in hexane as an eluent afforded 331 mg (80% yield) of compound I as a white powder. ESI-LC/MS (M+H).sup.+ =588; m.p. =199-200.degree. C. ##STR62##

Detailed Description Text - DETX (37):

To part H(1) compound (109 mg, 0.2 mmol) in 25 mL 2-necked round bottom flask (flame-dried and fitted with an argon-filled balloon, vacuum take-off and a thermocouple) was added 0.5 mL of DMPU (distilled over CaH.sub.2 under

reduced pressure, stored with 4A molecular sieves). The resulting slurry was warmed while stirring until becoming a clear solution, which was diluted with THF (1.5 mL). The reaction mixture was evacuated and purged three times with argon, then cooled to -78.degree. C. To the cooled reaction mixture was added dropwise 0.42 mL of a 0.5 M solution of LDA in THF.sup.1 (0.21 mmol). An amber colored solution formed. After stirring at -78.degree. C. for 30 minutes, a solution of Example 2 Part G aldehyde (67 mg, 0.26 mmol) in THF (0.5 mL) was added via a syringe. After addition, the resulting yellow solution was stirred at -78.degree. C. for 30 minutes, then at 0.degree. C. for 1 hour before quenched with an aqueous solution of ammonium chloride. The reaction mixture was extracted three times with ethyl **acetate** (10 mL). The organic extracts were combined, washed with water and brine, dried (MgSO.sub.4) and evaporated. The crude product was purified using flash chromatography on silica gel eluting with 5% EtOAc/hexane. The desired fractions were pooled and concentrated, and the collected residue dried in vacuo overnight to give Part H(2) compound as a white foam, 80 mg (68% yield).

Detailed Description Text - DETX (42):

To a cooled (0.degree. C.) solution of Part A compound (450 mg, 0.766 mmol) in anhydrous CH.sub.2 Cl.sub.2 (9 mL) under argon was slowly added trifluroacetic acid (886 mL, 12.5 mmol). After the addition was complete, the reaction mixture was allowed to stirred at 0.degree. C. for 10 minutes and at room temperature for 3 hours and then the solvent was removed in vacuo. The reaction mixture was quenched with phosphate solution (pH 7.5, 12 mL) and extracted with CH.sub.2 Cl.sub.2 (20 mL.times.2). The combined organic layer was washed with brine (saturated NaCl solution, 10 ml), dried over sodium sulfate (Na.sub.2 SO.sub.4) and filtered. The solvent was removed in vacuo. Flash silica gel chromatography using 30% to 50% ethyl **acetate** in hexane as an eluent afford 330 mg (91% yield) part B compound as a white powder. ESI-LC/MS (M+H).sup.+ =474, MP (.degree. C.)=253-254. ##STR67##

Detailed Description Text - DETX (53):

To a mixture of 4-fluoro-benzaldehyde (935.8 g, 7.54 moles) and methyl isobutyryl **acetate** (1087 g, 7.54 moles) was added piperidine (64.2 g, 0.75 mol), followed by acetic acid (22.6 g, 0.38 mol). The mixture was heated to 80 to 85.degree. C. for about 2 hours. 16 Liters (4.times.4L) of toluene was added and mixed with the reaction mixture. The toluene was removed using a rotavapor (50-65.degree. C./20-90 torr), leaving a yellow oil. The yellow oil was dissolved in 5 L MTBE and washed with: 1.times.3 L HCl (0.5N) 1.times.3 L NaHCO.sub.3 (saturated soln.) 1.times.3 L DI water

Detailed Description Text - DETX (56):

To a solution of the crude compound B (3078 g) in aqueous HOAc (16 L) was added ammonium **acetate** (1446 g), followed by cupric **acetate** monohydrate (1859 g). The reaction was refluxed between 120 to 124.degree. C. for 12-15 hours. Approximately 90% of the acetic acid was evaporated to produce a green slurry. The slurry was then mixed with 14 L MTBE.

Detailed Description Text - DETX (59):

To a 500 mL round bottom flask equipped with a magnetic stirrer and a nitrogen inlet was charged Part C compound (17) (Scheme 6) (50.0 g, 128.4 mmol) and toluene (170 mL). The mixture was stirred at 20-25.degree. C. until a clear solution was obtained. A solutin of 65% Red-Al in toluene (57.8 mL, 192.6 mmol) was added and the reaction mixture was heated to 80.degree. C. until complete as determined by HPLC. The reaction mixture was cooled to .about.20.degree. C. and quenched by pouring it into cold (0-5.degree. C.) 20% HCl (495 mL). Phases were separated and the spent toluene phase was discarded. The pH of the aqueous phase was adjusted from <0 to 4-5 with 10N NaOH. Ethyl **acetate** (500 mL) was added and the pH adjustment continued to 7-8. The phases were separated. The aqueous phase was extracted with additional ethyl **acetate** (2.times.500 mL). The combined rich ethyl **acetate** solution was washed with water (3.times.250 mL) and concentrated under reduced pressure to .about.465 mL. This solution was carried through to the next oxidation step.

Detailed Description Text - DETX (60):

The rich ethyl **acetate** solution was charged from above into a three neck 1-L flask equipped with mechanical stirring, temperature controller, and addition funnel and cooled to 0-5.degree. C. To the slurry, potassium bromide (1.53 g, 12.8 mmol) and TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) (0.20 g, 1.28 mmol) were added. The pH of NaOCl (sodium hypochlorite) solution (212.1 mL) was adjusted to 9.1 and added to the slurry at a rate such that the temperature remained at 0-5.degree. C. Stirring was continued at 0-5.degree. C. until the reaction was complete as determined by HPLC. The aqueous phase was extracted with EtOAc (2.times.200 mL). The combined rich organic phase was washed with a 1:1 solution of sat. aq. Na.sub.2 S.sub.2 O.sub.3 (sodium thiosulfate) (75 mL) and water (75 mL) followed by wash of the rich organic phase with 1N NaOH (250 mL). The rich organic phase was washed with water (250 mL) and concentrated to .about.100 mL under reduced pressure. Isopropanol (IPA) (400 mL) was added and the resulting mixture was heated to reflux (80-85.degree. C.). The solution was distilled to a volume of .about.250 mL. Water (50 mL) was added and the crystal slurry was stirred at 70-80.degree. C. for 1 h then allowed to cool to 20-25.degree. C. over at least 1 h. The slurry was held at 20-25.degree. C. for at least 1 h before collecting the solid by filtration on a Buchner funnel. The cake was washed with cold (0.degree. C.) IPA/water (4:1) (2.times.50 mL) and dried to a constant weight under vacuum at 40.degree. C. to afford 41.5 g (90%) of title aldehyde as a white crystalline solid.

Detailed Description Text - DETX (67):

A.sub.n N.sub.2 purged 250 mL 3-neck rb flask was charged with Example 35 pyridine derivative (18) (5.0 g, 13.9 mmol), Example 36 sulfone (16) (6.92 g, 15.3 mmol) and THF (75 mL). The stirred solution was cooled to -74 to -78.degree. C. Slowly a 1M solution of LIHMDS (lithium bis(trimethylsilyl)amide) (15.3 mL, 15.3 mmol) in THF was charged at a rate such that the temperature remained between -70 and -78.degree. C. After addition of the base was complete, the reaction mixture was warmed to .about.-45.degree. C. over .about.15 minutes. The stirred reaction was quenched at -70.degree. C. by slow addition of sat. aq. NH.sub.4 Cl (7.5 mL) solution and water (38 mL). The dry ice bath was removed and the solution was warmed to 20-25.degree. C. from the reaction mixture. Ethyl **acetate** (50 mL) was added, the mixture agitated, and layers separated. The organic layer was

washed with saturated sodium bicarbonate solution (2.times.38 mL) followed by brine (25 mL) and concentrated to a volume of 50 mL. Acetonitrile (50 mL) was added and the solution was concentrated to a volume of 50 mL. This step was repeated. Water (.about.5-6 mL) was slowly added to the hot solution (60-70.degree. C.) until the cloud point was reached. The thin slurry was held for 30 min at high temperature and then slowly cooled over several hours with stirring. The product was filtered, cake was washed with a 5:1 mixture of acetonitrile and water, and dried to afford 7.5 g (91%) of the title compound as a white crystalline material.

Detailed Description Text - DETX (73):

was transferred to a 5.0-liter round bottom flask equipped with a mechanical stirrer, a thermometer, and a septa. While temperature was controlled at <29.degree. C., 1 N HCl (aq) was added to the above aqueous layer until the pH=6.94. Subsequently, 330 mL of ethyl **acetate** was added to the aqueous layer followed by charging more 1 N HCl (aq) until pH=2.82. After separating and saving the ethyl **acetate** layer, the aqueous layer was extracted with ethyl **acetate** (330 mL.times.3). The combined ethyl **acetate** layers containing acid Ib.sup.8 of the invention: ##STR85##

Detailed Description Text - DETX (74):

were washed with 50% brine (265 mL), brine (427 mL), separated and mixed with a suspension of L-arginine (27.4 g, 157 mmol) in ethanol (276 mL) and water (138 mL). The mixture was evaporated to dryness under reduced pressure at ca 45-50.degree. C. To the resulting white solid were added ethyl **acetate** (450 mL), ethanol (316 mL), and water (145 mL) followed by heating the white suspension to 50.degree. C. Another 36.7 mL of water was added to dissolve all solids at 56.degree. C; subsequently 1720 mL of ethy **acetate** was added to the hot solution to initialize the crystallization. The white suspension was stirred at 50.degree. C. for 1.5 h and at ambient for 13 h. After filtration, the crystalline solid was washed with 143 mL of a mixture of EtOAc (200 mL), EtOH (12 mL) and H.sub.2 O (6 mL) and was dried in vacuo at 40-50.degree. C. for 24 h. The title product obtained as a white solid weighed 78.9 (g). Yield, 75.7%. [.alpha.].sup.25.sub.D =+23.0 (c 0.31, CH.sub.3 CN:H.sub.2 O, 1:1, v/v).